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FOWL PARALYSIS (NEUROLYMPHOMATOSIS GALLINARUM) IN CHICKS UNDER THREE MONTHS OF AGE[1]

BY JACOB BIELY[2], V. ELVIRA PALMER[3] AND I. MICHAEL LERNER[3]

Abstract

Fowl paralysis is generally considered to be a disease of birds over three months of age. In the present paper data are presented on 45 out of 244 chicks (18.4%), which developed clinical symptoms of fowl paralysis before 90 days of age. Of these, 20 were inoculated and 25 were not inoculated. Forty-two of the 45 chicks (93.3%) showed lesions in the nervous system. Nine chicks (20%) showed lymphomatous tumors.

Seventeen of the 45 cases (37.7%) occurred in chicks less than 60 days of age; the same number of cases occurred between 60 and 74 days of age; and 11 cases (24.4%) occurred between 75 and 89 days of age. The mean age of all chicks that developed paralysis was 64.4 days.

The fact that typical fowl paralysis occurred in one chick at 37 days, and in several from 40 to 44 days of age, would indicate that the disease may develop at a very rapid rate.

Fowl paralysis (*Neurolymphomatosis gallinarum*), although known to affect adult birds, is generally considered to be a disease of young birds. Thus it has been noted that spontaneous cases of clinical fowl paralysis may occur in fowls 3 to 18 months of age. The disease, however, appears to be prevalent in pullets which are approaching maturity or have just started to lay, the largest percentage of cases usually occurring between three and eight months of age.

Although the precise "incubation period" of fowl paralysis is not known, it is thought to be about three months. This figure is based on the evidence that spontaneous or experimental cases of fowl paralysis usually do not appear until the birds are about three months old.

Review of Literature

Pappenheimer, Dunn and Cone (11) record in detail the age incidence of fowl paralysis of the Leghorn flock at the Storrs Experimental Station during the years 1922, 1924 and 1925. In 1922 and 1924 the disease appeared after the

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birds were three months old, and in 1925 after they were four months of age. The disease continued to appear until the birds were 16 months old. The highest mortality was regularly encountered in the first five months after the appearance of the disease, or roughly, between the months of August and December. At the Storrs' egg laying contest, out of 3800 pullets which came under observation when about seven months old, 97 developed paralysis between 7 and 18 months of age. In partial histories obtained from several farm flocks it was determined that fowl paralysis might make its first appearance as early as ten weeks or as late as the fifth or sixth month of age.

Considerable variation in the age incidence of fowl paralysis has been also noted in the successful transmission experiments reported by Pappenheimer *et al.* Thus chicks, two to five days old, inoculated with the same material upon the same day, developed symptoms of fowl paralysis at different ages. The earliest clinical case was observed at 10 weeks of age, while the majority of the birds developed fowl paralysis between 110 and 205 days of age.

Kaupp (7) considers that birds from 4 to 12 months of age are most susceptible to fowl paralysis. Stafseth and Johnson (12) state that in Michigan fowl paralysis is the most serious ailment affecting birds from 4 to 14 months. Doyle (4) states that the first symptoms of paralysis usually appear when the birds are from three to six months of age, but birds under three, and more than eight, months of age may become affected with the disease. He makes the important observation that "the peculiar type of blindness, which has such a close flock association with paralysis, usually does not occur in birds much less than 8 months of age". The earliest age of occurrence of paralysis in the literature is recorded by Doyle, who reports that a cockerel showed clinical symptoms of fowl paralysis at 47 days of age. Thomas (13) reports that an outbreak of fowl paralysis started in a flock of chickens about two to four months old and reappeared when the birds were 10 to 12 months old. In another flock the disease appeared between the ages of three and five months, while in a third flock fowl paralysis appeared in pullets three to six months old. Emoto and Miyamoto (5) state that in Japan the earliest spontaneous case was observed at 4 months, and that birds about 12 months of age are the most frequently affected. Newsom (10) considers fowl paralysis to be a disease of "mature fowls, from 5 to 15 months of age". McGaughey (9) states that fowl paralysis usually first appears in young pullets from four to eight months. Beach (1) notes that fowl paralysis occurs in fowls between the ages of four and eight months, although it has been observed in pullets three months old and in hens up to 18 months of age. Beaudette and Hudson (2) report that fowl paralysis occurs most often between the third and eighth months of age. They introduce a table showing that during 1929-1930 and 1930-1931 cases of fowl paralysis were submitted every month of the year for diagnosis at the New Jersey Agricultural Experiment Station. Warrack and Dalling (14) state that fowl paralysis is found to occur mostly in young birds, from 8 or 9 weeks up to 18 months of age, but that "the most commonly affected birds are those from 3 to 5 months old". Johnson (6)

reports that fowl paralysis occurs most frequently in "young birds between 3 and 18 months of age". A few cases outside of these limits have been noticed. Mayhew (8) reports cases of paralysis in chicks as young as eight weeks, and as old as eight months of age. Twelve out of 27 cases developed before three months of age.

The purpose of this paper is to record observations on the incidence of fowl paralysis in chicks under three months of age.

Material and Methods

The chicks used for this study were hatched in several lots from eggs obtained from a Rhode Island Red and Black Orpington flock of birds in which heavy losses from fowl paralysis had previously occurred. When the chicks were 24 hours old they were leg-banded and transferred to battery brooders, where they were kept from eight to ten weeks. The chicks were then confined in small pens with wire floors, or pens with wooden floors with peat-moss litter. Throughout the experiment water and a balanced mash were kept before the chicks at all times.

The house in which the chicks were confined was isolated from other buildings and, with the exception of the attendant, was not visited by anyone. At the time the first lot of chicks was put in the battery brooders there were no paralyzed birds in the house. The premises were cleaned and disinfected before the experiment was started.

About one-half of the chicks (1-7 days of age) were inoculated by various routes with suspensions of tissue from the nervous system of birds affected with fowl paralysis. All chicks showing clinical symptoms of the disease were kept under observation for several days before they were killed and subjected to a careful post-mortem examination. With a few exceptions, gross lesions characteristic of fowl paralysis were found in the nervous system of affected birds. A number of birds also showed lymphomatous tumors. As, in the writers' experience, the typical lesions found in clinical cases of fowl paralysis always yielded the same histopathological picture, it was not deemed necessary to make a microscopic examination of every affected chick. However, histopathological studies were made of several birds to confirm microscopically the diagnoses made on the basis of clinical symptoms and lesions found on post-mortem examination.

Data

Out of 244 chicks forming the 10 lots under observation, 22 were killed or died from causes other than fowl paralysis. Their ages varied from 47 to 86 days, the majority being about 75 days old. None of these chicks showed lesions characteristic of fowl paralysis. On the other hand, 45 chicks (18.4%) developed clinical symptoms of the disease before they reached 90 days of age. Of these, 20 chicks were inoculated and 25 were not inoculated.

With the exception of four chicks which developed paralysis of the wing (Table I, Chicks Nos. 691, 324, 301 and 664), all the chicks showed typical paralysis of one or both legs. Two of the 45 chicks died and the rest were killed.

The results of the post-mortem examinations are presented in Table I, which shows that gross lesions were found in the nervous system of all but three chicks. Moderate to extreme changes were noted in the posterior root ganglia of the spinal cord in the thoracic region, in the corresponding segments of the spinal cord, in the brachial plexus, in the lumbosacral plexus,

TABLE I

DISTRIBUTION AND EXTENT OF GROSS LESIONS IN 45 CHICKS SHOWING CLINICAL SYMPTOMS OF FOWL PARALYSIS

Number of chick	Age in days when symptoms of paralysis were noticed	Lesions in lumbo-sacral plexus and sciatic nerves	Lesions in brachial plexus and posterior root ganglia in thoracic region	Lympho-matous tumors	Number of chick	Age in days when symptoms of paralysis were noticed	Lesions in lumbo-sacral plexus and sciatic nerves	Lesions in brachial plexus and posterior root ganglia in thoracic region	Lympho-matous tumors
675	37	++	+++	X	NN	66	++		
508	40		+	X	784	66	?	+	
517	42	++	++	X	977	69		+++	
704	44	+	++		2227	70	+	+	
677	44	+	++		324	70	++	+++	
649	47	+	+++	X	301	70	++	+++	
671	47	+++	+++		605	71	++	++	
308	50	+	++		524	71	+	+++	
508	52		+++		349	72	?	++	
781	52		+		450	72	++	?	
650	52	++	+		144	72	++	+	X
2225	55	+++	+++		664	73	+	++	
347	55	++	+++		772	76	++		
521	55		++		657	77	++	++	
778	57	++	++		879	77			
785	57	+	+	X	496	78	++	++	
304	58	++	+++		3702	78	++	+	
691	60		++		328	78		++	
864	62	++	+		518	80		++	
516	63		+		2397	81	+++	++	
865	65			X	48	82	++	++	X
769	65	+	+		869	84	?	?	X
					506	89	++	?	

NOTE:—?, *lesions doubtful;* +, *moderately thickened and translucent;* ++, *considerably thickened and translucent;* +++, *enormously thickened and translucent;* X, *lymphomatous tumors present.*

and in the sciatic nerves.* The distribution of the lesions according to size is shown in Table I. It will be seen that there was a preponderance of +++ and ++ lesions. Considering the fact that the chicks on the average were under 65 days of age, it is remarkable that such large lesions could develop in a comparatively short period of time without markedly influencing the rate of growth or the health of the chicks before the actual onset of paralysis.

**The gross lesions found in the brachial plexus and posterior root ganglia of the spinal cord in the thoracic region, and in the lumbosacral plexus and sciatic nerves, will be reported in the text as being found in the "brachial region" and the "lumbosacral region" respectively.*

From an examination of Table I it will be seen that gross lesions were found more often in the brachial region than in the lumbosacral region. This bears out the writers' general observation that gross lesions are more commonly found in the brachial region than in the lumbosacral region, in spite of the fact that clinical paralysis of the wings is much rarer than paralysis of the legs. Thirty-nine chicks (86.6%) showed gross lesions in the brachial region as compared with 31 chicks (69.1%) showing lesions in the lumbosacral region. Altogether, 42 of the 45 chicks (93.3%) showed lesions in either the brachial or lumbosacral regions or both. It should be further noted that 9 (20%) of the chicks showed lymphomatous tumors.

TABLE II

THE DISTRIBUTION OF 45 CASES OF FOWL PARALYSIS ACCORDING TO AGE

Age in days	Inoculated chicks		Non-inoculated chicks		Total	
	Number	%	Number	%	Number	%
35–39	—	—	1	4.0	1	2.2
40–44	2	10.0	2	8.0	4	8.9
45–49	2	10.0	—	—	2	4.4
50–54	1	5.0	3	12.0	4	8.9
55–59	3	15.0	3	12.0	6	13.3
60–64	1	5.0	2	8.0	3	6.7
65–69	3	15.0	2	8.0	5	11.1
70–74	3	15.0	6	24.0	9	20.0
75–79	3	15.0	3	12.0	6	13.4
80–84	1	5.0	2	8.0	3	6.7
85–89	1	5.0	1	4.0	2	4.4
Total	20	100.0	25	100.0	45	100.0
Mean	64.0 days		64.8 days		64.4 days	

Table II shows the actual and the percentage distribution of the 45 cases of fowl paralysis according to age. It will be seen that the earliest case occurred between 35 and 39 days of age, or to be exact, at 37 days (Fig. 1). Seventeen of the 45 cases (37.7%) occurred in chicks less than 60 days of age, the same number of cases occurred between 60 and 74 days of age, and 11 cases (24.4%) occurred between 75 and 89 days of age.

On the basis of the total number of chicks under observation (244), about 7% developed paralysis before 60 days of age; 7% between 60–75 days of age; and 4.5% between 75–90 days of age. The mean ages of the inoculated and non-inoculated chicks were 64.0 and 64.8 days respectively. The mean age of all the chicks which developed paralysis was 64.4 days.

FIG. 1. *Chick No. 675 at 37 days of age, showing clinical symptoms of fowl paralysis.*

Two of the 9 cases which showed lymphomatous tumors occurred in chicks under 2 months of age; in fact, one as early as 37 days of age. This is in agreement with the writers' previous observation (3) that the "incubation periods" of fowl paralysis and lymphomatous tumors are similar.

Discussion

The data show the very early age at which clinical cases of fowl paralysis may appear. As far as the authors are aware, this is the first time that a case of clinical paralysis occurring as early as 37 days of age is recorded. Although several investigators mention the fact that fowl paralysis may occur in chicks at two months of age, the condition is considered as exceptional. As already pointed out in the review of the literature, fowl paralysis is regarded as a disease affecting primarily chicks over three months of age. The data presented in this paper on the contrary show that a comparatively large number of cases of fowl paralysis may occur in chicks under three months of age.

In connection with the age incidence of fowl paralysis in the 45 chicks, there appears to be no consistency in the distribution (Table II). The means, standard deviations and coefficients of variation of the inoculated and non-inoculated chicks are as follows.

	Inoculated	Non-inoculated
Mean age	64.000 ± 1.986	64.800 ± 1.517
Standard deviation	13.155 ± 1.408	13.200 ± 1.254
Coefficient of variation	20.525 ± 2.286%	20.370 ± 2.012%

It would thus appear that inoculation of chicks at 1-7 days of age had no effect either on the mean age of the appearance of fowl paralysis or on the age distribution of the 45 cases.

The data in respect to age variation agree with those of Pappenheimer, Dunn and Cone (11) and of Warrack and Dalling (15), who report considerable variability in the time interval between inoculation and the appearance of clinical symptoms of fowl paralysis.

From an examination of Table I it will be seen that there does not appear to be any correlation between the "incubation period" of the disease and the extent of the gross lesions. Thus chick No. 675 showed correspondingly just as large lesions at 37 days as chick No. 2397 at 81 days. Furthermore, from the clinical observations it is evident that the degree of paralysis is not commensurate with the extent of the gross lesions as revealed by post-mortem examination. It is obvious, therefore, that symptoms of paralysis are only incidental and an external manifestation of an insidious disease. The fact that typical fowl paralysis with gross lesions may develop as early as 37 days of age suggests that the disease may attack chicks at a very early age and that the disease may develop at a very rapid rate.

Acknowledgment

The authors wish to express their great indebtedness to the following feed organizations for their generous contributions of feed and other supplies used in this and other studies of fowl paralysis at the University of British Columbia; The Brackman-Ker Milling Company, Limited; Buckerfield's, Limited; McLellan and McCarter, Limited; and The Vancouver Milling and Grain Company, Limited.

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SWINE ERYSIPELAS[1]

By J. S. Fulton[2]

Abstract

Swine erysipelas has been recognized in widely separated districts in Saskatchewan, the disease having appeared in the acute, sub-acute and chronic forms.

The organism has been isolated from a number of cases and positive serological reactions obtained in others.

The organism isolated has been used to inoculate healthy pigs and in this way the disease has been reproduced.

As a result of the evident high mortality in the swine herds of Saskatchewan, it was deemed advisable to make a systematic study of the diseases common to the province that we might determine the nature of the affections causing the heavy losses.

During the years this work has been in progress it has become ever more apparent that the number of deaths gives a very inadequate picture of the total losses which are principally due to the large percentage of unthrifty animals usually designated as "runts". Investigation has revealed the fact that many of our so-called runts suffer from a specific disease known as swine erysipelas, in which the correct diagnosis has not been made because of the lack, or variety, of symptoms presented by the infected animals.

History

Swine erysipelas was first studied by Pasteur and Thuillier in 1882, and although these investigators did not establish the etiology of the disease, they did contribute much to the information available at that time.

The causative organism of swine erysipelas was isolated by Löffler in 1885.

Occurrence

Swine erysipelas occurs in all European countries, certain territories being heavily infested, so that outbreaks occur with regularity each season. The disease is most prevalent during the summer months, it abates towards fall, while only sporadic cases are observed during the winter.

Cause

The causative organism of swine erysipelas, *Erysipelothrix rhusiopathiae*, is a short, very slender, straight or slightly curved bacillus. In suitable liquid media, such as serum broth, the organism appears in long branching forms; to the naked eye, growth in this medium appears at first slightly cloudy, later granular and, as coalescence of the individual granules occurs, the growth falls to the bottom leaving the supernatant liquid clear.

[1] *Manuscript received January 23, 1933.*
Contribution from the Animal Diseases Laboratory, University of Saskatchewan, Saskatoon, Canada.

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Types

Swine erysipelas presents a great variety of clinical types usually classified as acute, chronic and cutaneous. All of these have been observed in Saskatchewan, together with a sub-acute type presenting symptoms which in severity are midway between those of the acute and chronic. The chronic form is the most prevalent in Saskatchewan.

The Acute Form

In this type death generally occurs in a few days and the symptoms—fever, vomiting and lassitude, are sufficiently definite to permit a clinical diagnosis to be made.

The Chronic Form

Here diagnosis is difficult, either because of the lack of definite symptoms, or because of the great variety. It is so insidious in onset that it may be present in a herd for some time before any suspicion arises that there is anything wrong. The litter develops well until the pigs are two or three months old, when they cease to put on weight in spite of good food and care. The skin loses that bright, clean appearance indicative of good health and assumes a dirty, dry condition, while the hair becomes long and shaggy. We have found that pigs about six months old, suffering from the chronic form of swine erysipelas, often weigh no more than 35 to 50 lb., while healthy animals on the same farm weigh 200 lb. at the same age. Too often in the past these cases have been considered due to parasitism and the true cause has never been determined. In many of the chronic cases no more definite symptoms than those described above are presented and are so indefinite that diagnosis is impossible without laboratory tests, consequently such cases are unsuspected and become a prolonged source of infection.

Together with this unthrifty appearance some infected individuals show a dry, crusty eczema over the back, on the ears and sometimes around the lower joints of the limbs.

Erysipelothrix rhusiopathiae has evidently a decided affinity for joints, as in sub-acute or chronic swine erysipelas the infection often becomes localized in the joints of the limbs, setting up an acute or chronic arthritis. No difficulty has been experienced by the writer in isolating the causative organism in pure culture from such joints.

In cases where arthritis is acute there is a marked stiffness, and if forced to exercise, the animals show evidence of great pain. Should the joints of both hind limbs become involved the animals lose control of the hind quarters so that the condition is often described as paralysis, the true cause never being determined. It is usually the stifle, or hip joint, or both which are affected when complete loss of control of the hind limbs occurs.

In these cases the synovial sac is much enlarged and distended with fluid, which is turbid in appearance. The membrane is greatly thickened and

villus processes hang from the surface. The inner lining also shows numerous outgrowths, parts of which become detached and may be found floating in the fluid (joint mice).

The tissue surrounding the joint is usually edematous and this condition may extend a considerable distance from the focus of infection. In one case where the hip joint was involved the edema was readily discernible from the joint down to a point halfway between the stifle and the hock. This type of arthritis is usually confined to swine suffering from sub-acute erysipelas and although most animals ultimately succumb they may live for a considerable time.

Chronic arthritis may follow the acute but is often chronic from the beginning. Both in experimentally produced and in field cases of chronic swine erysipelas primary chronic arthritis has been observed. In these cases little pain or stiffness is evidenced until the process has developed to such an extent that the entire bony structure of the joint is changed and its free movement greatly impaired.

The bones of the carpal and tarsal articulations are more often affected than those forming the other joints of the body. The lesions observed have varied from a slight roughening of the free surface of one or two of the bones to marked outgrowths affecting all the bones comprising the joints.

In the more advanced cases, even although ankylosis does not occur, the joint may be rendered quite immobile through the interlocking of the bony outgrowths from the various bones.

The membrane of the synovial sac and the tissue surrounding the joint may be somewhat thickened but there are no marked changes such as are apparent in the acute type of arthritis already described. The synovial fluid may be more viscid than that found in normal joints but it is usually quite clear in appearance.

Endocarditis

Hutyra and Marek (1) state that chronic erysipelas "occurs most frequently as chronic erysipelatous-endocarditis in animals which have recovered from the acute affection. After the disappearance of the acute manifestations the pigs are usually lively and have a good appetite for a time, but on careful observation it may be noticed by comparison that they are stunted in their development."

In Saskatchewan chronic erysipelas has appeared in herds where the acute form never did occur and it has been reproduced experimentally by inoculating healthy pigs with cultures of *Erysipelothrix rhusiopathiae* secured from the joints of chronic field cases. The experimental animals so treated did not show any suggestion of acute erysipelas, but several weeks after inoculation they began to lose weight, assume an unthrifty appearance and later developed joint lesions from which a pure culture of the causative organism was recovered.

Endocarditis, which has been described as a characteristic lesion in chronic swine erysipelas, is not at all constant in the primary chronic cases we have examined. The valves in some instances have been slightly thickened, but vegetations have not been observed. Cultures made from the valves which appeared to be abnormal have yielded negative results.

Other Effects

The most constant change observed from gross examination of the organs of animals affected with chronic swine erysipelas is the enlargement of the spleen. This organ is usually dark in color and its surface is studded with elevations varying from the size of a pea to that of a large bean.

Microscopically the spleen shows marked congestion. The blood vessels are greatly distended and the spaces within the pulp are filled with red and white blood cells; the Malpighian bodies become indistinct.

The glomeruli of the kidney show marked congestion, the epithelium of the tubules is disintegrated and in some areas there is an excess of connective tissue.

Foci of granular degeneration appear throughout the liver tissue, interspersed with areas which are fairly normal.

Differential Diagnosis

The possibility of confusing parasitism and swine erysipelas must always be borne in mind especially in these cases of chronic erysipelas where no definite symptoms are presented.

In cases where chronic arthritis develops, the lower joints of the limbs may become so enlarged as to give the impression that the long bones are curved and that the animal is affected with rickets.

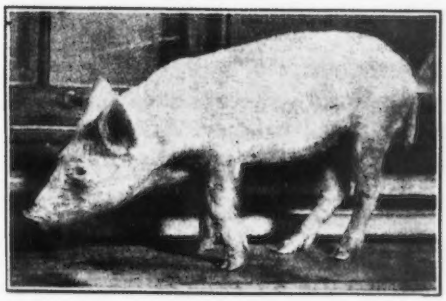

FIG. 1.

FIG. 2.

FIG. 1. *Pure-bred Yorkshire, three months old, suffering from chronic swine erysipelas. Weight, 17½ lb. Erysipelothrix rhusiopathiae isolated from synovial fluid of the left carpal articulation.* FIG. 2. *Osteo-arthritis of carpal joints of pigs affected with chronic erysipelas. (Photographs by R. K. Baker).*

The dry, crusty eczema, so common in animals infected with *Erysipelothrix rhusiopathiae*, may be quite readily confused with mange or sun scald. During the course of this investigation we have not attempted making a diagnosis of swine erysipelas without either isolating the causative organism or making use of serological tests on animals which could not be destroyed.

Tests of the Organism

The writer wishes to acknowledge a very complete report on two cultures submitted to Dr. J. B. Buxton, Institute of Animal Pathology, Cambridge. These cultures, secured from the joints of pigs suspected of having chronic swine erysipelas, have been compared with an authentic strain of *Erysipelothrix rhusiopatiae*, and apart from minor differences, have been found to be indistinguishable.

The strain described as "Runc", with which they were compared, was isolated from a pig suffering from the acute form of swine erysipelas and was pathogenic for mice in a dose of 0.00001 cc., while 24-hr. broth cultures from the original seed material of the Saskatchewan strains were lethal in doses of 0.1 cc., but not with smaller amounts. After passage through pigeons, however, the Saskatchewan strains, known as "Latta" and "Graham", became much more virulent, in fact quite comparable to the "Runc" strain. The same quantity (0.00001 cc.) of the 24-hr. broth culture, inoculated intraperitoneally, killed mice in four and six days respectively.

Dr. Buxton also carried out serum protection experiments using a fixed dose of anti-erysipelas serum with varying amounts of virulent cultures. This serum gave protection to mice receiving 10,000 minimum lethal doses of the "Latta" culture, 1000 of the "Graham" and 1000 of the "Runc" strains. Controls, receiving the same quantity of normal horse serum as was given of the anti-erysipelas serum, did not survive. Other controls receiving no serum also died. These results were confirmed by the application of serological tests *in vitro*.

Acknowledgment

The author wishes to acknowledge personal communications from L. T. Giltner, Pathological Division, Bureau of Animal Industry, Washington, D.C. which proved of great assistance in carrying out the work in the early stages of the investigation.

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THE PARASITIC HELMINTHS OF CANADIAN ANIMALS

I. THE CESTODARIA AND CESTODA[1]

By Robert Arnold Wardle[2]

Abstract

One adult Cestodarian, 53 adult Cestoda and 24 larval Cestoda are known to occur in Canadian animals. The majority of the Cestoda are distributed among the Dibothriocephaloidea and Taenioidea.

Of the Cestoda, 29 of the adult species are represented in Europe, 14 are recorded elsewhere only in the United States, 6 are recorded only in Canada and 4 are unassignable. Of the 24 larval forms, 14 and one doubtful form are also European, 3 and two doubtful forms are recorded elsewhere only in the United States, 2 occur also in Japanese waters and 2 are not assignable.

An attempt is made to evaluate the economic status of the cestodes of the area.

Introduction

The scantiness of published information concerning the internal parasites of Canadian animals has hitherto precluded the preparation of a comprehensive report upon the incidence of endoparasitic helminths in the area. A considerable range of cestodarian and cestodan forms has now been recorded, however, and this information, supplemented by an accumulation of observations made by the writer and his coworkers upon the cestodes and nematodes of fishes, birds and mammals, appears sufficient to justify the presentation of the following survey. No claim towards completeness can be made. The cestodes of the domesticated mammals and birds and of the wild game animals are scarcely known. Very little information is available concerning the nematode parasites of any host group, and no information at all is available concerning the Acanthocephala.

The report may be of value, however, in directing the attention of other workers in parasitology to those fields in which further information is desirable, and in stimulating further investigations in this important branch of applied zoology.

The classification of the Cestodaria and Cestoda adopted here is, in broad outline, that proposed by Southwell (53) but the two groups will be regarded as distinct classes of the Platyelmia and no attempt will be made to subdivide them into orders. The Cestodaria comprise two families, the Cestoda comprise 30 families. These families are all familiar to the parasitologist, and diagnoses of them and of their component genera have been published by Meggitt (36) so that they need not be defined here. In order to keep the list of literary references within bounds only the more recent and comprehensive references that concern each superfamily will be alluded to. The references given under the heading of each species will be the Canadian references only.

[1] *Manuscript received January 18, 1933.*
Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man.

[2] *Professor of Zoology, University of Manitoba.*

No reference will be made to questions of technique as an account of the principal methods used in the study of cestode material has recently been published by the writer (63).

Taxonomy

Class CESTODARIA Monticelli, 1892

Of the two families which comprise this class of Helminthes, the ***Amphilinidae*** are not represented in Canadian records, and the ***Gyrocotylidae*** are represented by one species only. No comprehensive monograph on the class is available, but a taxonomic survey of the genus *Gyrocotyle* has been published by Dollfus (12), and gyrocotylid material from Californian waters has been described by Kofoid and Watson (26), by Watson (67) and by Ward (58).

Fam. GYROCOTYLIDAE Benham, 1901

Gyrocotyle urna Grübe et Wagener, 1852. Not uncommon in the intestine of *Hydrolagus colliei* in the Straits of Georgia, B.C. Usually two twin individuals are present in each infested host. *Gyrocotyle fimbriata* Watson, 1911, appears to be synonymous with this species. *Reference:* Wardle (60).

Class CESTODA s. str.

Superfam. DIBOTHRIOCEPHALOIDEA Stiles, 1906

Although the generic term *Dibothriocephalus* Luhe, 1899, is now generally superseded in nomenclature by *Diphyllobothrium* Cobbold, 1858, there is still some doubt as to whether the two genera are synonymous and the point can only be decided by a re-examination of material from the dolphin species from which Cobbold obtained his *Diphyllobothrium stemmacephalum*. If they are synonymous, the superfamily designation should of course be changed to Diphyllobothrioidea but in the meantime the term originally suggested by Stiles may be allowed to stand.

There is no comprehensive publication dealing with the whole superfamily. The families Caryophyllaeidae, Cyathocephalidae, and Amphicotylidae have been partially monographed by Nybelin (44); the Caryophyllaeidae have also been monographed by Hunter (24). Keys to the species of the genus *Diphyllobothrium* have been provided by Meggitt (37) and Baylis (2), and the biology of *D. latum* in North America has been discussed by Essex (17), Magath (33, 34), Vergeer (57), Ward (59) and others, in addition to the Canadian references given. The dibothriocephaloid tapeworms of eastern Canada and of Arctic Canada have been recorded by Cooper (6–11), those of western and northern Canada by Wardle (60, 61, 62, 64). Of the eight families constituting the superfamily, all, except the Echinophallidae, are represented by one or more species in the area.

Fam. CARYOPHYLLAEIDAE Müller, 1787

Glaridacris catostomi Cooper, 1920. Common in the intestine of species of *Catostomus*, probably over the whole area; adults and larvae may occur in the same individual host, the adults free in the gut lumen, the larvae imbedded in pits in the mucosa. Recorded from *Catostomus catostomi*, Lake Waskesiu, Sask., and Lake Simcoe, Ont. *Reference:* Wardle (61).

Fam. CYATHOCEPHALIDAE Lühe, 1899

Diplocotyle olrikii Krabbe, 1874. Common and numerous in the intestine of *Salvelinus alpinus stagnalis*, *Salvelinus fontinalis*, *Leucichthys artedi*, *Coregonus clupeaformis*, *Coregonus atikameg*, *Myoxocephalus quadricornis*, in Hudson Bay, Wakeham Bay, Churchill River, James Bay. In *Salvelinus malma*, Bernard Harbor, N.W.T.

It appears to be typically a parasite of circum-arctic Salmonoidea, but has been recorded in *Pseudopleuronectes americanus* at St. Andrews, N.B. The species *Bothrimonus intermedius* Cooper and *Diplocotyle nylandica* Schneider are in the writer's opinion merely synonyms of *D. olrikii*. *References:* Cooper (9, 11), Wardle (61).

Cyathocephalus truncatus (Pallas, 1781). Not uncommon in the intestine of fresh-water salmonoids, probably over the whole area east of the Rocky Mountains. Recorded from *Coregonus clupeaformis* and *Leucichthys zenithicus*, Lake Winnipeg; from *Salvelinus alpinus malma*, Spray Lakes, Alberta; from *Cristivomer namaycush*, Bernard Harbor, N.W.T.; and from *Coregonus clupeaformis*, Lake Ontario. The species *C. americanus* Cooper appears to be synonymous with *truncatus*. The plerocercoid larva occurs in the amphipod species *Pontoporeia affinis* (Lindström). *References:* Cooper (9, 11), Wardle (61)

Fam. DIBOTHRIOCEPHALIDAE Lühe, 1902

Subfam. 1. LIGULINAE Lühe, 1899

Ligula intestinalis (Linn. 1758). Common as a plerocercoid larva in the body cavity of fresh-water fishes across the area. The writer has specimens from *Catostomus*, *Notropis*, *Micropterus*, *Gasterosteus*, *Salmo*, *Perca*, varying in length from 20 to 600 mm. The adult has been recorded in Canada only from *Mergus americanus*, Shuswap Lake, B.C., and in the United States only from *Mergus* sp. and *Mergus merganser*, although reported in Europe from a wide range of water birds. *References:* Cooper (9), Wardle (62).

Schistocephalus solidus (Müller, 1776). Not uncommon as a plerocercoid larva in the body cavity of fresh-water fishes. It has been thus reported in *Cottus* sp., Shuswap Lake, B.C.; *Uranidea formosa*, Port Credit, Ont.; *Gasterosteus bispinosus*, Chamcook Lake, N.B.; *Pygosteus pungitius*, Bernard Harbor, N.W.T. The writer has also examined material from *Salvelinus fontinalis*, in Big Bent, Pumpkin Seed and Commandant lakes, Quebec.

The adult has been recorded in Canada only from *Mergus americanus*, Nanaimo, B.C., and as lying free on the shore of Nanaimo Lake, B.C. In the United States it has been recorded only in *Lophodytes cucullatus*. *References:* Cooper (9, 11), Wardle (62).

Subfam. 2. DIBOTHRIOCEPHALINAE Lühe, 1902

Diphyllobothrium latum (Linnaeus, 1758). Common but localized in distribution, in man, dog, bear, mink, cat, in Manitoba, possibly in Saskatchewan. Recorded in 81% of 500 dogs in the vicinity of Lake Winnipeg in 1931; in 4% of vagrant cats in Winnipeg in 1930-31. Procercoid larva in *Diaptomus oregonensis*. Plerocercoid larva in epaxonic and hypaxonic musculature, and, rarely, on peritoneal gut surface, of *Esox lucius*, *Lucioperca vitreum*, *Perca flavescens*, *Lucioperca canadense;* mean number of larvae per fish ranges from 2.3 in *Esox* to 1.2 in *L. canadense*. The adult worm agrees closely in structural features with European and Japanese material of *D. latum* and it seems well established that Canadian material is either this form, imported from Europe, or an indigenous subspecies identical morphologically with it. *References:* Nicholson (41, 43), Wardle (61, 64).

Diphyllobothrium canadense Cooper, 1921. Recorded from intestine of Northern Raven (*Corvus corax principalis*) at Bernard Harbor, N.W.T. Possibly synonymous with *D. cordiceps* Weinland, recorded by Linton (29) from *Pelicanus erythrorhynchus*, Yellowstone Lake, Wyoming. *Reference:* Cooper (11).

Diphyllobothrium cordatum (Leuckart, 1863). Recorded from the bearded seal (*Erignathus barbatus*) at Bernard Harbor, N.W.T., together with ***Diphyllobothrium lanceolatum*** (Krabbe, 1865) and ***Pyramicocephalus phocarum*** (Fabricius, 1780). The larval form of the latter species has been recorded by the writer from *Gadus ogac*, Richmond Gulf, Hudson Bay. *References:* Cooper (11), Wardle (61).

Diphyllobothrium sp. *larvae inquirendae*. The term is used to imply larvae to which the crucial test of rearing in a suspected adult stage host has not been applied, so that they cannot be definitely assigned to any known species. Seven such diphyllobothriid larvae are known from Canadian hosts, namely:—

1. Free in gut of *Salvelinus marstoni*, Bernard Harbor, N.W.T. *Reference:* Cooper (11).

2. Free in gut of *Erignathus barbatus*, Bernard Harbor, N.W.T. Possibly the larva of *D. lanceolatum* (Krabbe, 1865). *Reference:* Cooper (11).

3. Free or encysted on the peritoneal gut surface and coelomic surface of *Oncorhynchus nerka kennerlyi* in Kootenay and Chilliwack lakes, B.C. Common and numerous. Possibly identical with the form described by Fasten (19) from Cutthroat trout in Klause and Cooper lakes, Washington, and from *Oncorhynchus tschawytscha* and *O. nerka kennerlyi* in Klause Lake, Washington. *Reference:* Wardle (60).

4. Free on peritoneal gut surface of *Oncorhynchus kisutch*, Straits of Georgia, B.C. Possibly identical with the form described by Linton (29) in *Salmo mykiss*, Yellowstone Lake, Wyoming, as the larva of *D. cordiceps*. *Reference:* Wardle (60).

5. Encysted in liver of *Lota lota maculosa*, Lake Winnipeg; uncommon. *Reference:* Wardle (61).

6. Free or encysted on the peritoneal gut surface of *Salvelinus fontinalis*, Wakeham Bay, Ungava. Numerous in host. *Reference:* Wardle (61).

7. Embedded in liver of fingerling *Salvelinus fontinalis*, Yarmouth, N.S., and inducing abdominal ascites; one larva per host. No previous record.

Fam. PTYCHOBOTHRIIDAE Lühe, 1902

Bothriocephalus scorpii (Müller, 1776). Not uncommon in the pyloric caeca of shore-frequenting teleostean fishes in Atlantic and Pacific coastal waters of the area. It has been recorded from *Hemitripterus americanus*, St. Andrews, N.B.; from *Myoxocephalus quadricornis*, Bernard Harbor, N.W.T.; from *Leptocottus armatus*, *Enophrys bison*, *Hexagrammos decagrammus*, *Hexagrammos superciliosus*, *Myoxocephalus polycanthocephalus*, *Apodichthys flavidus*, Nanaimo, B.C. *References:* Cooper (9, 11), Wardle (60).

Bothriocephalus occidentalis (Linton, 1898). Recorded from the pyloric caeca of *Leptocottus armatus* and *Sebastodes* spp., Nanaimo, B.C. Uncommon. *Reference:* Wardle (60).

Bothriocephalus cuspidatus Cooper, 1921. Common in the gut of freshwater teleosts over the whole area east of the Rockies. It comprises probably a group of subspecies, such as *B. c. cuspidatus* in *Lucioperca vitreum*, *B. c. hiodontos* in *Hiodon* spp. and *B. c. luciopercae* in *Lucioperca canadense*. The life history in Minnesota waters has been elucidated by Essex (18). *References:* Cooper (8, 9), Wardle (61).

Bothriocephalus claviceps (Goeze, 1782). Recorded in *Anguilla rostrata*, Chamcook Lake, N.B. *Reference:* Cooper (9).

Clestobothrium crassiceps (Rudolphi, 1819). Common and numerous in *Merluccius bilinearis*, St. Andrews, N.B., and *Merluccius productus*, Nanaimo, B.C. *References:* Cooper (9), Wardle (62).

Fam. TRIAENOPHORIDAE Nybelin, 1922

Triaenophorus tricuspidatus (Bloch, 1779). Common in gut of *Esox lucius* over the whole area. Plerocercoid larvae encysted in musculature of coregonid fishes and in the liver of young *Esox* and *Perca*. The species *T. nodulosus* (Pallas) and *T. robustus* (Olsson) are regarded by the writer as synonyms of *tricuspidatus*. *References:* Cooper (9), Nicholson (42), Newton (40), Wardle (61).

Fam. AMPHICOTYLIDAE Nybelin, 1922

Subfam. 1. AMPHICOTYLINAE Lühe, 1902.

Eubothrium oncorhynchi Wardle, 1932. Not uncommon in the pyloric caeca of *Oncorhynchus* spp. Straits of Georgia, B.C.; 3–10% of fishes are infected during the summer months. Usually one strobila per host, occasionally two, rarely more. *Reference:* Wardle (60).

Eubothrium crassum (Bloch, 1779). Recorded in pyloric caeca of *Salvelinus alpinus stagnalis*, Hudson Bay. Common and numerous. In intestine of *Myoxocephalus quadricornis*, Churchill River; in *Salmo salar*, Miramichi River, N.B. Common. *References:* Wardle (61), Kuitunen-Ekbaum (27).

Eubothrium salvelini (Schrank, 1781). Recorded in the pyloric caeca of *Salvelinus fontinalis*, James Bay; *Salvelinus alpinus malma*, Spray Lakes, Alberta; *Cristivomer namaycush*, Clear Lake, Manitoba, Lake Manitoba, Lake Huron, Lake Temagami, Lake Ontario. Probably coexistent with *Salvelinus* and *Cristivomer* across the area. *References:* Cooper (9), Wardle (61), Kuitunen-Ekbaum (27).

Eubothrium rugosum (Batsch, 1786). Common in the pyloric caeca of *Lota lota maculosa*, lakes Winnipeg, Huron, Ontario. Dimorphic, with *conformatus* type and *deformatus* type. *References:* Cooper (9), Wardle (61, 65), Kuitunen-Ekbaum (27).

Subfam. ABOTHRIINAE Nybelin, 1922

Abothrium gadi Van Beneden, 1871. Intestine of *Melanogrammus aeglefinus*, Passamaquoddy Bay, N.B. and Bay of Fundy, N.S., *Gadus callarias*, Campobello Island, N.B. *Reference:* Cooper (9).

Fam. HAPLOBOTHRIIDAE Cooper, 1914

Haplobothrium globuliforme Cooper, 1914. Recorded from *Amia calva* at Go-Home Bay, Muskoka, Ont. *References:* Cooper (6, 9).

Superfam. TETRARHYNCHOIDEA Southwell, 1930

No comprehensive monograph on this superfamily has yet been published and the classification is chaotic. Reference may be made to the publications of Dollfus (13, 14, 15), Guiart (21, 22), Pintner (45–48) and Southwell (52, 53). The classification adopted here is a modification of that suggested by Guiart which has the disadvantage that it is based primarily upon larval characters, so that any adult form whose larval stage is unknown, must necessarily remain *incertae sedis*. The Canadian tetrarhynchoids have been completely neglected. Linton (30) has described however from the adjacent New England waters, *Otobothrium crenacolle* and a number of created species of the now discredited genera *Rhyncobothrium*, *Synbothrium* and *Tetrarhynchus*.

Fam. SPHYRIOCEPHALIDAE Guiart, 1927

No Canadian records.

Fam. DIBOTHRIORHYNCHIDAE Dollfus, 1931

Dibothriorhynchus grossus (Rudolphi, 1810), *larva inquirenda*. Recorded from body cavity of *Gadus callarias*, Port Burwell, Ungava. *Reference:* Wardle (61).

Fam. TENTACULARIIDAE Dollfus, 1931

Nybelinia surmenicola Okada, *larva inquirenda*. Common and numerous in the stomach of *Ophiodon elongatus*, and the epaxonic musculature of *Theragra* spp., Straits of Georgia, B.C. *References:* Wardle (60, 66).

Fam. VAULLEGEARDIDAE Guiart, 1927

No Canadian records.

Fam. LAKISTORHYNCHIDAE Guiart, 1927

Grillotia erinacea (Van Beneden, 1857). Recorded as adult in the intestine of *Hexanchus caurinus*, Straits of Georgia, B.C., and as a larva in the musculature of *Theragra* spp., same locality. *Reference:* Wardle (62).

Fam. EUTETRARHYNCHIDAE Guiart, 1927

No Canadian records.

INCERTAE SEDIS

Gilquinia squali (Fabricius, 1793). Common and numerous in the intestine of *Squalus sucklii*, Straits of Georgia, B.C. *Reference:* Wardle (60).

Superfam. PHYLLOBOTHRIOIDEA Southwell, 1930

This superfamily has been partially monographed by Southwell (50). Of the two families recognized by this author, the ***Oncobothriidae*** are not as yet represented in Canadian records, and the ***Phyllobothriidae*** are represented by a single larval form, probably in an abnormal host. Linton (30), however, has recorded 10 phyllobothriid and 13 oncobothriid species from fishes in the adjacent coastal waters of New England, and there is little doubt that a survey of selachian fishes in Canadian waters would yield a number of phyllobothrioid forms.

Fam. PHYLLOBOTHRIIDAE Braun, 1900

Phyllobothrium salmonis Fujita, 1922, *larva inquirenda*. Common in either encysted or free condition in the alimentary tract of *Oncorhynchus* spp., especially *O. gorbuscha* in British Columbia coastal waters. The adult has been recorded under the name of *Phyllobothrium keta* by Canavan (5) from a specimen of *O. keta* in Alaskan waters. *Reference:* Wardle (60).

Superfam. LECANICEPHALOIDEA Southwell, 1930

Fam. LECANICEPHALIDAE Braun, 1900

No Canadian representatives of this family have been recorded but Linton (30) has recorded *Discocephalum pileatum* in species of *Carcharinus* in New England waters.

Superfam. PROTEOCEPHALOIDEA Southwell, 1930

The North American species of this family have been monographed, up to date of publication, by La Rue (28), and a criticism of the characters used in distinguishing proteocephaloid genera and species, with a key to the species known at the date of publication, has been published by Meggitt (38). The known Canadian species belong to the genus *Proteocephalus*, Weinland, 1858 (= Ichthyotaenia Lonnberg, 1894) and to the genus *Corallobothrium* Fritsch, 1866. No members of the family Monticellidae have been recorded in the area.

Fam. PROTEOCEPHALIDAE La Rue, 1911 (= Ichthyotaeniidae Ariola, 1899)

Proteocephalus coregoni Wardle, 1932. Recorded in intestine of *Coregonus atikameg*, Hudson Bay. *Reference:* Wardle (61).

Proteocephalus laruei Faust, 1920. Common and numerous in the intestine of *Coregonus clupeaformis* and *Leucichthys zenithicus*, Lakes Winnipeg and Waskesiu; in *Leucichthys hoyi*, Lake Winnipeg; in *Coregonus clupeaformis*, Lake Ontario. The common proteocephalid of coregonid fishes in the area. *Reference:* Wardle (61).

Proteocephalus pinguis La Rue, 1911. Recorded in the intestine of *Esox lucius*, Lakes Winnipeg and Winnipegosis. The common proteocephalid of *Esox* in the area. *Reference:* Wardle (61).

Proteocephalus arcticus Cooper, 1921. Recorded from *Salvelinus marstoni*, Bernard Harbor, N.W.T.; from *Salmo clarkei* fingerlings, Departure Bay creek, Vancouver Island; from *Oncorhynchus kisutch* fingerlings, Cultus Lake, B.C. *References:* Cooper (11), Wardle (62).

Proteocephalus ambloplitis (Leidy, 1887). Recorded by Cooper as adult in the intestine of *Micropterus dolomieu*, and as larval in the body cavity of the same host, Georgian Bay, Lake Huron; noted by the writer in the intestine of *M. dolomieu*, Whitefish Lake, Quebec, both in adult and larval stages; and as a larval stage only in *Ameiurus nebulosus*, same locality. *Reference:* Cooper (7).

Corallobothrium fimbriatum Essex, 1927, *larva inquirenda*. Common in the intestine of *Ameiurus nebulosus*, Lake Winnipeg, about six larvae per host. *Reference:* Wardle (61).

Superfam. TAENIOIDEA Zwicke, 1841

Eleven families of taenioid cestodes are recognized by Southwell (53), and to these may be added the families *Diploposthidae* Poche and *Biuterinidae* Meggitt. Representatives of only seven of these families have been recorded in the area, and the forms recorded here bring the total number of recorded Canadian taenioids from 11 to 28. There can be little doubt that this meagre total is not fully representative of the distribution of this superfamily in the area and that the forms recorded in domesticated mammals and birds, and in many of the migratory wild birds, in the United States will eventually be found also in Canada.

The taenioid species in the United States have been recorded by Stiles and Hassall (56), Stiles (55), Ransom (49), Hall (23), Mayhew (35), Douthitt (16), Linton (31), Millzner (39) and others.

Fam. TAENIIDAE Ludwig, 1886

Multiceps multiceps (Leske, 1780). Recorded as isolated proglottides from the dogs of the Canadian Arctic Expedition, 1913–18, at Collinson Point, Alaska. *Reference:* Cooper (11).

Multiceps serialis (Gervais, 1847), *larva inquirenda*. Common in *Lepus americanus* in western Canada in the form of cysts, $\frac{1}{2}$–$2\frac{1}{2}$ in. in diameter, in the subcutaneous tissue, each cyst having several hundred scolices. Percentage of infestation among 400 rabbits in Manitoba was 15.6 in 1931. The maximum number of cysts per host was 14. *Reference:* Boughton (3).

Taenia hydatigena (Pallas, 1776). Found by Riddle (unpub.) in 4 out of 70 vagrant cats in Winnipeg, 1931-32. To this species the writer would refer also a *larva inquirenda* common in the muscles of *Alces alces* in Alberta.

Taenia pisiformis (Bloch, 1780), *larva inquirenda*. Common as transparent vesicles in the liver and coelom of *Lepus americanus*, Manitoba and Saskatchewan. Boughton records an incidence of 14.7% in 400 rabbits from Manitoba, 1931, the average number of cysts per host being 20, the maximum number per host being 106. *Reference:* Boughton (3).

Taenia solium (Linnaeus, 1758). There are apparently no records of this cosmopolitan parasite of man in Canada, although there is no doubt of its occurrence in the area. The writer is informed by veterinary surgeons that the larva is much less common in hogs than was formerly the case. The writer has material from Saskatchewan hogs.

Taeniarhynchus saginatus (Goeze, 1782). There appear to be no published records of this form in the area but the writer has larval material from western Canadian cattle. It is probably a common parasite of man in the area.

Fam. ANOPLOCEPHALIDAE Cholodkowsky, 1902

The North American species of this family, recorded prior to 1915, have been described by Douthitt (16). The European forms have been mono-

graphed by Baer (1). Of the four subfamilies recognized by Douthitt, the Linstowinae and Avitellinae have not been recorded in the area.

Subfam. ANOPLOCEPHALINAE Blanchard, 1891

Cittotaenia pectinata americana Douthitt, 1915. Common in young *Lepus americanus* in Manitoba. Boughton records 100% of infestation in rabbits 3–8 weeks old between June and November but practically no infestation after the beginning of November, when the animals commence to feed largely upon bark, and an infestation of only 0.26% in rabbits more than one year old. *Reference:* Boughton (3).

Moniezia expansa (Rudolphi, 1805), ***Moniezia alba*** (Perroncito, 1897), ***Moniezia planissima*** (Stiles and Hassall, 1893). Described as common in lambs in eastern Canada. *Reference:* Stevenson (54).

Subfam. THYSANOMINAE Fuhrmann, 1907

Thysanosoma actinioides Diesing, 1834. Described as occurring in the bile ducts of lambs in eastern Canada. *Reference:* Stevenson (54).

Fam. DAVAINEIDAE Fuhrmann, 1907

Davainea comitata Ransom, 1909. Recorded from Yellow-bellied Sapsucker (*Sphyrapicus varius varius*), Tamaracouta, Quebec, in the abdominal cavity. *Reference:* Lloyd (32).

Fam. HYMENOLEPIDIDAE Railliet et Henry, 1909

A monographic study of the North American species of this large family has been published by Mayhew (35). Reference may be made also to the publications of Ransom (49) and Linton (31). Of the five subfamilies recognized by Mayhew, the *Oligorchinae* and *Diorchinae* are not represented in Canadian records.

Subfam. HYMENOLEPIDINAE Ransom, 1909

Weinlandia planestici Mayhew, 1925. Occurring in intestine of *Planesticus migratorius*, Saskatoon. This, and *W. microcirrosa* are probably widely distributed over western Canada in the American robin.

Weinlandia corvi Mayhew, 1925. In *Corvus brachyrhynchos hesperis*, Saskatchewan. Common. Three specimens per host.

Subfam. FIMBRIARINAE Fröhlich, 1802

Fimbriaria intermedia Fuhrmann, 1903. Recorded from intestine of the Pacific Eider-duck (*Somateria v-nigra*) at Bernard Harbor, N.W.T. *Reference:* Cooper (11).

Fimbriaria fasciolaria (Pallas, 1781). The writer has material from *Mergus americanus*, Nanaimo, B.C. It has not previously been recorded in the area though probably widespread and common. Linton (31) reports it in *Mergus serrator*, on the New England coast.

Subfam. APLOPARAKSINAE Mayhew, 1925

Aploparaksis sp. Recorded from *Somateria v-nigra*, at Bernard Harbor, N.W.T. *Reference:* Cooper (11).

Fam. DILEPIDIDAE Railliet et Henry, 1909

This large family of bird-infesting cestodes is probably more widely distributed in the area than appears to be indicated by the ten species recorded here.

Subfam. DILEPIDINAE Railliet et Henry, 1909

Lateriporus geographicus Cooper, 1921. Recorded as present in stomach of *Somateria v-nigra*, Bernard Harbor, N.W.T. *Reference:* Cooper (11).

Lateriporus sp. To this genus the writer would refer a collection of small cestodes, too fragmentary to identify specifically, taken from the intestine of a Dipper (*Cinclus mexicanus*) at Taft, B.C.

Anomotaenia constricta (Molin, 1858). Common and numerous in intestine of *Corvus brachyrhynchos brachyrhynchos*, in Manitoba.

Anomotaenia sp. The writer would refer to this genus a number of small cestodes from the Willett (*Catoptrophorus semipalmatus*), Saskatoon, which, owing to the partial obliteration of the rostellar armature, cannot be specifically identified. They are cestodes up to 100 × 1.5 mm. in dimensions with segments campanulate when mature, to longi-rectangular when gravid, in shape, and markedly salient. The scolex is approximately 0.3 mm. in length and its rostellum has one circle of hooks. The testes lie completely behind the germarium and vitellarium and number 36. The genital pores are alternate and marginal, one-fifth of the proglottid length from the anterior border. The eggs occur in packets of 4–8 apparently in the parenchyma.

Subfam. DIPYLIDINAE Stiles, 1896

Dipylidium caninum (Linnaeus, 1758); ***Dipylidium sexcoronatum*** Ratz, 1900; ***Dipylidium compactum*** Milzner, 1926; ***Dipylidium gracilis*** Milzner, 1926; ***Dipylidium diffusum*** Milzner, 1926. All found by Riddle (unpub.) in *Felis domesticus*, Winnipeg. Common. Numerous. Incidence of infestation among 72 cats was 25%.

Subfam. PARUTERININAE Ransom, 1909

Anonchotaenia sp. The writer would refer doubtfully to this genus two immature and damaged cestodes from *Dendroica aestiva*, Saskatoon. Ransom (49) has recorded *A. globata* from *Dendroica striata*.

Fam. ACOLEIDAE Ransom, 1909

The writer would refer to this family some fragments of a broad, fleshy cestode obtained from *Limnodromus griseus*, Alberta, which, although lacking a scolex, is undoubtedly acoleid in morphology. It may be noted that *Acoleus vaginatus* (Rudolphi, 1819) is recorded by Ransom (49) in the related host species *Himantopus mexicanus*.

Fam. TETRABOTHRIIDAE Linton, 1891

Tetrabothrius cylindraceus (Rudolphi, 1819). Common in intestine of *Larus argentatus*, Nanaimo and Deep Bay, Vancouver Island.

Discussion

Of the 53 species of Cestoda reported in the foregoing pages, 6 are at present known only in Canadian hosts; of the remaining species, 29 occur also in northwestern Europe, 14 are recorded elsewhere only in the United States, and 4—the ones referable to genus only—might be either European or United States in co-distribution. It may be noted that the six Canadian forms belong to genera occurring in Europe and are specifically very similar to European species. Of the 24 larval cestodes reported, 14 and one doubtful form are also European, 3 and two doubtful forms occur also in the United States, 2 occur also in Japanese waters, and 2 are unassignable.

The species which are coincident with European species appear to be more restricted in host distribution in North America than in Europe. While this may be more apparent than real, since fewer host species have been examined in this area, yet it appears to hold good for those species which, even in Canada, have been very fully studied. *Diphyllobothrium latum*, for example, has never been recorded in North America from salmonoid fishes, in striking contrast to the host distribution in Europe and Japan; *Triaenophorus tricuspidatus* in Canada is restricted in larval distribution to coregonid fishes whereas in Europe it has been recorded in pike, perch, ling, trout, grayling, catfish, popefish, salmon, etc.; *Cyathocephalus truncatus*, as an adult, is more restricted in host species in North America than in Europe and both adults and larvae of *Ligula intestinalis* and *Schistocephalus solidus* are similarly more restricted. Further it may be noted that North American forms which coincide with European species, show commonly certain morphological differences, not always sufficient to justify specific separation, although there is a tendency among North American workers towards such separation.

The inference might be drawn, therefore, although it is weakened by the paucity of information concerning North American cestodes, that North America has received most of its cestode species from Eurasia.

The genus *Proteocephalus*, however, seems definitely more American in possible origin than European. Of 44 species distinguished by characters other than dimensional ones, and reviewed by Meggitt (38), 19 are Old World in distribution, 25 are North American.

In any evaluation of the economic status of the Cestoda of the area, there are two viewpoints to be considered; namely, (1) the influence of cestode infestation upon the health of the host, and (2) the influence of such infestation upon the commercial value of the host.

Although possible injury to the host may be produced by a cestode in many ways—by occlusion of the gut lumen, by abstraction of the products of digestion, by intoxication of the host tissues with excretory products, by pressure upon a physiologically important organ—yet actual proved cases of such injury are few. Admittedly the pathology of wild animals remains almost completely unstudied and many cases of physiological injury to a host animal might pass unnoticed, but so far as parasitological experience goes, there is no evidence that enteric infestation with cestode parasites is seriously inimical to a *mature* animal. The intestine of fresh-water fishes is commonly so blocked with strobilae as to occasion surprise that the animals can gain any advantage whatever from their alimentary functions, yet in the examination of many thousand such fishes the writer cannot recall a single case of obvious malnutrition in a mature fish attributable to cestode infestation.

On the other hand, there is considerable evidence to show that such infestation may provoke malnutrition and nervous disturbance in an *immature* animal. There is considerable epidemic mortality in Canadian hatcheries among fingerling trout and salmon, attributable to gut occlusion with such cestodes as *Cyathocephalus* and *Eubothrium*, and such victims show all the external signs of malnutrition.

Nor can the common textbook suggestion that host animals may be injured by toxic excretions from cestode parasites, be supported by known facts. *Diphyllobothrium latum* is commonly asserted to be provocative of pernicious anaemia in the human host but Magath (33) from an analysis of a large number of such cases in the Mayo Clinic is disposed to deny any causal relation between tapeworm infestation and primary anaemia. It must be emphasized that there is no experimental evidence in favor of the possibility of adult cestoda excreting haemolytic waste products. The known facts concerning cestode physiology are scanty in the extreme but such information as is available suggests that the primary nutritive requirement of the cestode is glucose and that the cestode excretions are such as would be produced by glucose degradation. Brand (4) has demonstrated in the case of *Moniezia expansa*, that carbon dioxide, succinic acid, lactic acid and higher fatty acids are excreted into an artificial medium such as Ringer's solution, under anerobic conditions and at mammalian body temperature. There is no reason to regard such products as particularly toxic to a host animal, and the inimical effect of cestode infestation upon growing animals would appear to be due to the direct abstraction of the products of digestion or direct interference with the liver functions, rather than to intoxication by excretory products.

There is however a considerable mass of evidence to support the view that larval cestodes, somatic rather than enteric in host location, may bring

about serious disturbance by direct interference with the physiological efficiency of the organs in which they are located. Well-known and well-established examples of such pathogenicity are of course provided by the larval stages of *Echinococcus granulosus* in man, the so-called "hydatid cysts"; by the provocation of hepatic sarcoma in rats by the larva of *Taenia fasciolaris;* the inhibition of egg production in *Micropterus dolomieu* by larval *Proteocephalus ambloplitis;* the clogging of the atrium of the heart, or of the large blood sinuses of coregonid fishes by larval *Schistocephalus solidus;* the pathological changes in the gastric mucosa noted by the writer (Wardle, 62) in the Pacific fish *Ophiodon elongatus* when infested heavily with larval *Nybelinia surmenicola;* the abdominal dropsy and associated mortality of fingerling salmonid fishes in eastern Canadian hatcheries, when infested with a diphyllobothriid larva embedded in the liver tissue.

On the other hand, fresh-water fishes intensely infested with intermuscular larvae of *Diphyllobothrium latum* or *Triaenophorus tricuspidatus* do not seem unduly inconvenienced nor do they show any external morphological characteristics of such infestation. Boughton (3) was unable to associate the intense infestation of *Lepus americanus* by larval *Taenia pisiformis* and *Multiceps serialis* with the fluctuations in abundance of this host animal.

Although there is no reason to regard cestode infestation as particularly dangerous to human health, there is naturally somewhat of a prejudice towards foodstuffs which are infested with larval cestodes, and the influence of such infestation upon the commercial value of the infested animal is by no means negligible.

Johnstone (25), in reference to the seizure of marketable marine fishes by food inspectors at Liverpool, owing to their infestation with larval *Grillotia erinacea*, asserts that although in this case such infestation is harmless to the human consumer "the inspectors probably acted in the interests of public health in condemning such articles of food as contained obvious cyst-like structures in the flesh, as to the precise nature of which they were ignorant, since there is the possibility that these bodies might be detrimental to the health of those eating them. Further, the presence of these cysts was very obvious and rather unpleasant to the eye and might easily have prejudiced a customer against a particular vendor or fish".

A similar but much more serious example of the commercial importance of what might be called "aesthetic prejudice" is afforded by the infestation of coregonid fishes, especially the genus *Leucichthys*, in North America, by the larval stages of *Triaenophorus tricuspidatus*. The adult worm appears specific to *Esox*, possibly to *Cristivomer* and is therefore most unlikely to be capable of establishment in a warm-blooded host. Experimental tests by the writer suggest convincingly that this cestode cannot develop in the dog or in man and that it cannot withstand for more than a brief period, either the mammalian range of body temperature or the concentration of mammalian gastric juice. Nevertheless the rigid inspection tests imposed by the United States health authorities upon Canadian coregonids intended for consumption

in the United States—whilst unjustifiable upon scientific grounds and unfortunate in their inimical effect upon a thriving fresh-water fishing industry —cannot be severely criticized from the standpoint of public health, except as erring on the side of severity, since there is no question that a cisco heavily infested with the large, yellow bladders of the larval cestode, which when injured exude an unpleasant pus-like fluid, is not only most objectionable aesthetically but is most likely to prejudice the consumer against any kind of Canadian fish.

The redfish or Kokanee (*Oncorhynchus nerka kennerlyi*) of certain lakes in British Columbia is commonly so heavily infested with diphyllobothriid larvae that if this fish were of commercial value its sale on the public market would not be tolerated by any competent public health authority, although it is extremely probable that the infesting larvae can only develop further in certain water birds. It is in fact fortunate for the extensive salmon fishing industries that this infestation is restricted to the landlocked variety of Pacific salmon and does not occur in the marine species.

The aesthetic aspect of helminth infestation, the natural human prejudice against food infestation by "worms" is in the writer's opinion a factor far more important in the economic evaluation of the cestodes of an area than is the somewhat remote possibility of an animal being infested with some helminth that is directly communicable to man.

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TETRAMERES CRAMI SP. NOV., A NEMATODE PARASITIZING THE PROVENTRICULUS OF A DOMESTIC DUCK IN CANADA[1]

By W. E. Swales[2]

Abstract

A duck obtained from a farm near Ottawa, Canada, was found on post-mortem examination to be parasitized by a hitherto unreported nematode. No males were discovered but an examination of the nine females obtained revealed certain characteristics which appeared to justify the formation of a new species, for which the name *Tetrameres crami* is proposed.

In April, 1932, a duck obtained from a farm in the vicinity of Ottawa, Ontario, Canada, and used for a short period for experimental purposes at the Animal Diseases Research Institute, Hull, Quebec, was found on post-mortem examination to be parasitized by a hitherto unreported nematode.

The walls of the proventriculus were found to contain several small dark objects which proved, on dissection, to be nematodes deeply embedded in the crypts of Lieberkühn. In this way, nine examples were obtained.

Examination of the live specimens showed that they were members of the genus *Tetrameres* Creplin, 1846. Unfortunately, in spite of a careful search, no males were discovered.

The worms were fixed and preserved at the time of collection but a taxonomic study was delayed until recently, when through the courtesy of Dr. E. A. Watson, the Director of the Animal Diseases Research Institute, the helminthological collection from that laboratory was placed at the disposal of this Institute.

A study of the females thus collected revealed certain characteristics which, in spite of the absence of males, appeared to justify the formation of a new species. For this, the name *Tetrameres crami* is proposed in recognition of the work of Dr. Eloise Cram, which has so greatly facilitated the study of avian parasites in North America.

Tetrameres crami, sp. nov.

Male. Unknown.

Female. Body globular to sub-globular, 1.5 mm. to 2.3 mm. long by 1.2 mm. to 1.7 mm. wide, strongly furrowed longitudinally, the furrows corresponding to the median and lateral lines. The cuticle is also strongly striated transversely. The cephalic and caudal extremities protrude from the body mass; each is conical in outline.

The buccal capsule is almost circular, measuring 14 μ long by 12 μ wide, and is strongly cuticularized. The muscular oesophagus is well developed

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but is shorter than in the other members of the genus, being 0.16 mm. to 0.18 mm. long. It is surrounded near its middle by a prominent nerve ring, immediately behind which is situated the large excretory pore. Only one cervical papilla was seen in the type specimen, situated 72 μ from the anterior extremity. The oesophagus is nearly a millimetre long, penetrating the body mass to join the large sacular intestine which, owing to its distention with detritus, is seen as a dark irregular mass. The rectum is well defined and is 75 μ long; the anus is 0.15 mm. from the tip of the tail.

The vulva is 0.25 mm. from the tip of the tail and the ovejector is large with a somewhat barrel-shaped terminal portion, 40 μ long by 29 μ wide, with a simple small vestibule; there is no copulatory receptaculum present. The uteri and ovarian tubules are very long, their coils practically filling the body cavity. The eggs are thick-shelled and oval, measuring 45 to 48 μ long by 16 to 18 μ wide.

Remarks. This species differs in many respects from the other known North American species, *T. americana* Cram, 1927, being more closely related to *T. coccinea* (Seurat, 1914) and *T. cochleariae* Travassos, 1917. It differs from the former chiefly in the length of the muscular oesophagus, the relative positions of the anus and vulva, and in the dimensions and form of the ovejector. In comparison to the latter, it is smaller in almost every respect.

Hosts. Primary: Anas boschas domestica.

Secondary: Unknown, probably the same as that of *T. fissispina*, *Daphnia pulex* being very common in the locality.

Location. Proventriculus, the females embedded in the crypts of Lieberkühn, the males probably in the lumen.

Distribution. Canada (Ottawa, Ontario).

This is the first record of a member of the Family Tetrameridae being found in Canada, and according to Cram (2) American reports probably deal wholly with *T. americana.*

The Muscular Oesophagus of Tetrameres

It will be noted that in the above description the anterior portion of the oesophagus was not described as a pharynx, as it has been in other descriptions of related species. A pharynx, as defined by Baylis and Daubney (1), is an organ resembling a buccal capsule but having an exterior muscular coat. The anterior portion of the oesophagus in *T. crami*, *T. americana* and other species cannot be said to resemble a buccal capsule and the strongly muscular structure in two species examined is apparently interspersed with glandular tissue.

Although Travassos (6) in his illustration of *T. fissispina* does not show the nerve ring, his measurements indicate that it surrounds the so-called pharynx approximately at its middle. All other descriptions of known species

describe and show the position of the nerve ring similarly; this fact alone would indicate that this organ is a portion of the oesophagus and not a pharynx. The posterior portion is distinctly more granular and may readily be regarded as the glandular portion of the oesophagus; in fact, both portions more nearly answer the description of the oesophagi of the Physaloptera (5).

Pending further histological studies it is proposed that the "pharynx" of Tetrameridae be recognized as the muscular oesophagus and the rest of the organ as the glandular oesophagus.

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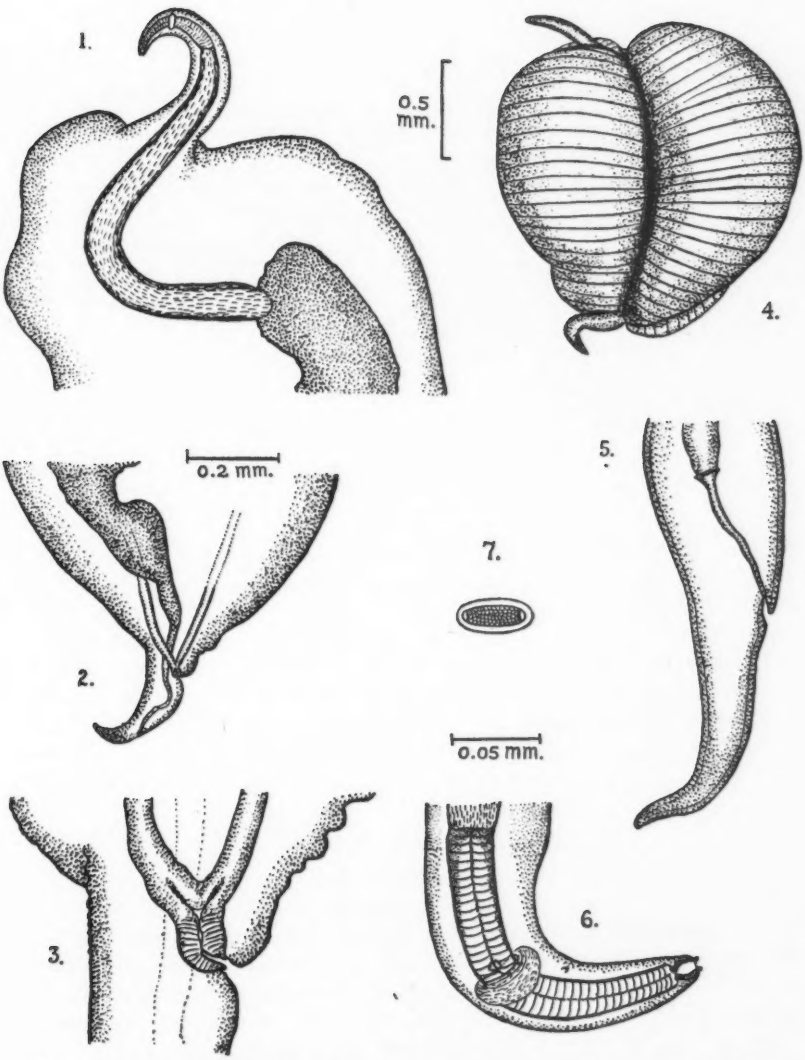

FIGS. 1–7. *Tetrameres crami, sp. nov. (1) Anterior part of female. (2) Posterior part. (3) Terminal portion of ovejector. (4) Female in toto. (5) Tail end of female, showing rectum. (6) Head end, dorsal view. (7) Egg, immature.*

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A ROOT ROT OF SWEET CLOVER AND RELATED CROPS CAUSED BY *PLENODOMUS MELILOTI* DEARNESS AND SANFORD[1]

By G. B. Sanford[2]

Abstract

The occurrence, hosts and symptoms of a hitherto undescribed root rot of *Melilotus*, *Medicago* and *Trifolium*, and the relation of temperature and the reaction of substrate to growth of the pathogen *Plenodomus meliloti* are discussed, and its pathogenicity demonstrated. It is suggested that the disease be called "brown root rot".

Evidence is that the hosts mentioned are susceptible only during the winter and early spring dormancy stage. Normal roots of sweet clover, when frozen at −4° C. for four days and subsequently kept at 2–3°, 9° and 16° C., did not become susceptible. The brown root-rot disease is distinct from true winter injury resulting from insufficient hardiness to cold.

The temperature range for vegetative growth and pycnidia of *P. meliloti* is from 0° to 27° C., with optimum between 15° and 17° C. Increasingly good growth occurs from 2° C. to optimum temperature. Severe lesions are produced at 2–3°, 9° and 16° C. The optimum pH value for growth in potato dextrose decoction is about 6.2, the other limits being approximately pH 3.2 and 8.2. Soils with an alkaline reaction apparently are unfavorable.

The disease is characterized by brown lesions, on or within which are an abundance of black to dark brown pycnidia. These bodies, 0.5 to 2 mm. in longest diameter, may have one or more spore bearing chambers. Each chamber may have one to several ostioles, through which the one-celled spores, averaging $5.2 \times 2.84\ \mu$, exude. The hyphae do not bear spores.

Dissemination of the pathogen by seed would not seem to be of practical importance. Control by crop sanitation is recommended, at least until varieties more resistant are available.

When observing types of winter injury of *Melilotus*, *Medicago* and *Trifolium* in Alberta and Saskatchewan, it occurred to the writer that certain soil-inhabiting micro-organisms might, while the plant was still in the dormant or semidormant condition, seriously increase such injury. As a result of studies begun early in 1926, it is now possible to report, in some detail, a hitherto undescribed root rot of these crops caused by *Plenodomus meliloti* (1). Newton and Brown (5) probably refer to the disease described herein; however, the causal organism was not isolated by them. The writer (7, 8) has also referred briefly to this root rot.

Description of the Disease

The disease is characterized by brown, slightly sunken, necrotic lesions, which appear on the tap or lateral roots and rootlets of sweet clover, alfalfa, and common clover (*Trifolium pratense*). Sometimes the lesions are more common on the roots level with the debris of a former crop. These lesions develop rapidly in all tissues of the host, particularly the pith, as soon as the surface soil thaws in the spring, or even during late winter if the weather is

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Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, in co-operation with the University of Alberta. The major part of this paper was presented at the meeting of the Canadian Phytopathological Society, Ottawa, December, 1929.

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mild. Plate I, Fig. 1, illustrates fairly well, the types of lesions which commonly occur on sweet clover roots under field conditions. Any part of the root surface may be affected (Plate I, Fig. 2), although the tips of the roots or smaller rootlets often succumb first. These lesions increase in various degrees, and frequently involve the lower two-thirds or three-quarters of the root system. Sometimes a part of the root system may be left intact (Plate I, Fig. 1, *A*). Some crown shoots are nearly always produced, even though the tap root is nearly destroyed (Plate I, Fig. 1, *C*). Often plants, the tap roots of which are severely rotted, but not near enough to the crown to be fatal, survive and partly recover by the aid of new roots produced near the crown. However, such cases usually result in more or less stunted plants. The progress of even severe lesions is terminated by callus when good root development and new growth begin. Since the lesions are dark brown, and, also, usually separated from the sound tissue by a decidedly light to dark brown marginal deposit unlike wound cork (Plate I, Fig. 6), it is suggested that the disease be called "brown root rot".

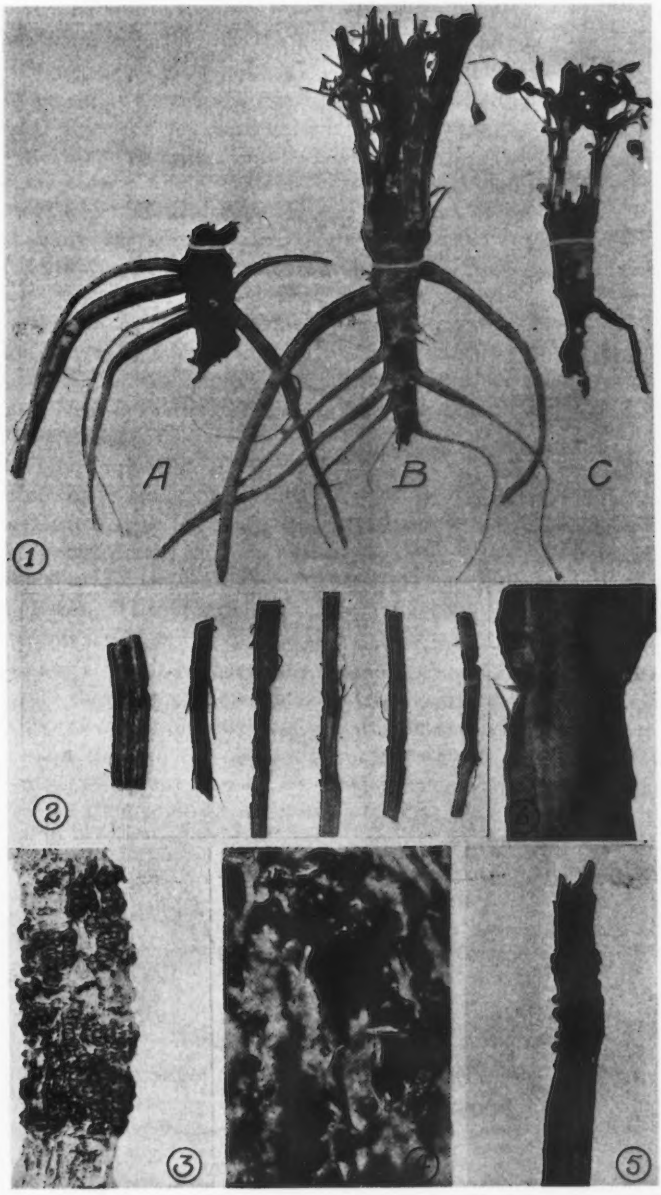
These characteristics rather definitely distinguish the brown root rot from Sclerotinia root rot, the lesions of which are softer and typically lack color. Usually pycnidia are very abundant on the surface (Plate I, Fig. 3) of the lesions of brown root rot, and within the necrotic tissue (Plate I, Fig. 4). These are dark brown to black, very closely clustered, and visible to the unaided eye. Sometimes only a few pycnidia, or even none, are obvious. They appear to be produced more abundantly on the lesions formed early, than on those of later incidence. Pycnidia may also occur on the crown portion above ground of dead host plants, although this is not common (Plate I, Fig. 5).

Distribution

Brown root rot has been observed on sweet clover, alfalfa, and common clover growing in the southern and central parts of Alberta and Saskatchewan, as far north as Prince Albert in the latter province, and Athabasca and Beaverlodge in Alberta. The fungus is native and apparently abundant in the cultivated black soils of the prairie area mentioned. The disease has not been reported as occurring elsewhere in Canada, or in foreign countries.

Economic Importance

In the spring of 1926, when the writer began to study this disease, it was prevalent in hundreds of acres of sweet clover which had been "winter-killed", more or less completely, in the area mentioned. In many stands, practically all the plants, regardless of their size, were severely lesioned, and bore an abundance of pycnidia of the fungus associated with brown root rot. The severity of the disease varies greatly from season to season, apparently being influenced by the environment as well as by the physiology of the host, but this is not well understood at present.



FIGS. 1 TO 6. *Types of lesions and the location of pycnidia associated with brown root rot on Melilotus, under field conditions. 1. A, the tap root rotted first; B, typical lesions on tap and lateral roots (this plant would recover); C, weak, ephemeral crown-shoots on root practically dead. 2. Various types of penetration. 3. Pycnidia characteristically clustered on surface of a lesion. 4. Pycnidia within root tissue. 5. Pycnidia on crown of dead root. 6. The dark brown marginal layer which usually separates the lesion from adjacent sound tissue.*

General Description of the Fungus

P. meliloti may be isolated easily from the pycnidia, from lesions next to the sound tissue, or from spores which are produced in the pycnidia. It grows well on a wide range of solid or liquid media, including ordinary potato dextrose agar, sterilized ground oat hulls, corn meal, rice, sterilized potato plugs, and sterilized roots of the host plants. Like many other fungi, the size, shape and color of the mycelium varies greatly according to the kind of substrate, and other factors, such as age, extremes of acidity, or the by-products of metabolism of associated fungi and bacteria. On ordinary potato dextrose and other carbohydrate media, the color of the mycelium, in mass, is light gray to ashen. Microscopically, young hyphae are hyaline and closely septate, but, aged or massed, they become brown with thicker walls. The width of the mycelium varies from 2 to 5 μ, and the cross walls are from 10 to 14 μ apart.

Dark, coriaceous pycnidia are produced abundantly in ordinary light, or in darkness, on a wide range of artificial media, and on the host. They are characteristically superficial on solid media (Plate II, Fig. 1), and on the necrotic tissues of the host plant (Plate I, Fig. 3). They also occur within the tissues (Plate I, Fig. 4) of the host, or develop submerged in solid or liquid media. On the host they are usually regularly subspherical and single, but on artificial media frequently confluent. In longest diameter they range from 0.5 to 2 mm. The initiation of the pycnidia follows closely the growth of the mycelium.

Spore-bearing chambers begin to form when the pycnidium is about 15 days old. As a rule these are initiated about six cells below the upper surface. Ordinarily, spores are produced when the pycnidium is about 50 days old, and these escape through the ostioles from 30 to 50 days later. However, the initiation of the spores may be hastened considerably, and the time for their escape from the pycnidium reduced to 60 days, by incubating pycnidia, 35 days old, in Knop's solution. The spores are hyaline, one-celled, and average 2.8 by 5.2 μ. A pycnidium may have one to several chambers, and as many as 12 or more ostioles. A single chamber may have one to several ostioles, through which the spores escape as a yellow exudate (Plate II, Fig. 1). The morphology of this species will be described more fully in a separate paper.

The Relation of Temperature to Growth

To determine the relation of temperature to growth of *P. meliloti*, the fungus was grown in Petri plates on ordinary potato dextrose agar. Ten of these cultures were incubated at each of the following temperatures: 0–1°, 2–3°, 7–8°, 12°, 15°, 18°, 21°, 23°, 25.5°, and 27° C. The diameters of the colonies on the plates, growing from a small, uniform disk of inoculum, placed at the centre of each plate, were recorded at intervals up to 10 days. The results are indicated by the curve in Text-fig. 1, the cardinal temperatures being 0°, 15–16°, and 27° C. The temperature relation to growth of *P. meliloti*

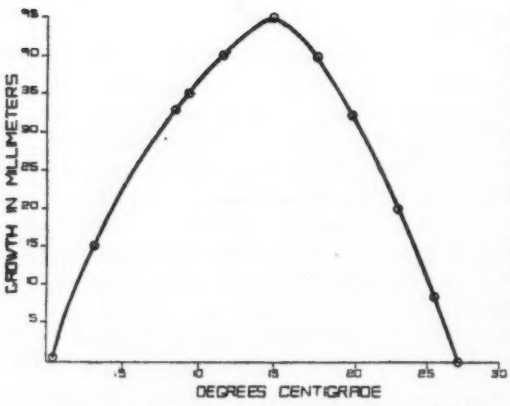


FIG. 1. *The curve indicates the growth of Plenodomus meliloti on potato dextrose agar at temperatures from 0° to 27° C.*

furnishes at least one striking contrast to *P. destruens* Harter, the optimum of which Harter (2) found to lie between 21.9° and 30.2°C., the minimum, close to 12.6° C., and the maximum at about 37.3° C.

Pycnospores, fresh from the pycnidium, when placed on microscope slides containing a thin covering of potato dextrose agar, and held at 15° C., germinated abundantly in 48 hr. At 1° and 4° C. superficial growth appeared from spores in ten and seven days, respectively, and at 10° in four days. Slight germination of the spores, but no further growth, occurred at 27° C.

The Relation of the pH Value of the Substrate to Growth

The substrate for this study was the standard potato dextrose decoction, strained, and adjusted to different initial pH values by adding N hydrochloric acid to the acid series, and $N/2$ sodium hydroxide to the alkaline series. Each Erlenmeyer flask, containing 100 cc. of the decoction, prepared as mentioned, was inoculated with a loop of spore suspension of *P. meliloti*, and incubated at 15° to 17° C. for 25 days. The pH values of the media in the various series were determined electrometrically at the outset, and every five days thereafter. On the 15th, 20th and 25th days, the dry weight of the fungus was determined, in addition to the pH values of the decoction. The growth of the fungus was ascertained by weighing the dried fungus-mat. The weights and pH values given are the average results from four flasks These are listed in Table I, and depicted in Text-fig. 2.

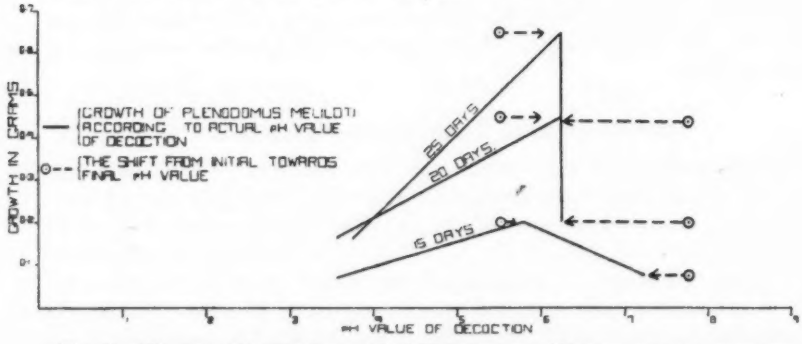
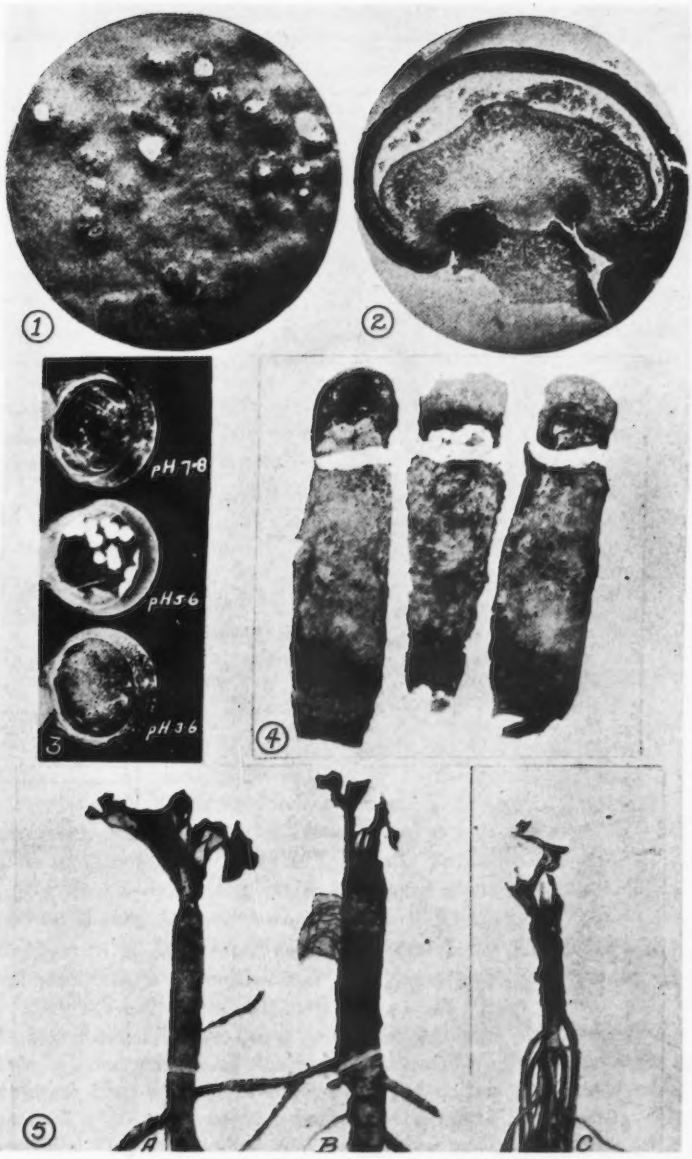


FIG. 2. *The curve indicates the growth of Plenodomus meliloti in potato dextrose decoction at different initial pH values and temperature 15° to 17° C.*



FIGS. 1 TO 5. *Views of pycnidia and vegetative growth of P. meliloti and of brown root rot experimentally produced on roots of Melilotus and Medicago. 1. Vegetative growth and pycnidia, on nutrient agar, showing ostioles and spore exudate. 2. A section through a pycnidium showing spore-bearing chamber. 3. The high per cent germination of spores but restricted growth of colonies, in potato dextrose decoction at pH 3.6, compared to lower per cent germination but freer growth of colonies at pH 7.8. 4. Vegetative growth and pycnidia, which developed in pure culture, on nutrient agar, buried in the soil, during winter and early spring. 5. Brown root rot experimentally produced by placing inoculum against the sound surface of A and B, a lateral and tap root, respectively, of Melilotus, and C, a tap root of Medicago.*

TABLE I

GERMINATION AND SUBSEQUENT GROWTH OF *Plenodomus meliloti* IN POTATO DEXTROSE DECOCTION AT VARIOUS pH VALUES

Media series	Initial	5 Days	10 Days	15 Days		20 Days		25 Days		Remarks
	pH	pH	pH	pH	gm.	pH	gm.	pH	gm.	
I	1.6	1.6	1.6	1.6	.0	1.8	.0	1.8	.0	No growth
II	3.6	3.6	3.6	3.6	.073	3.6	.152	3.7	.152	Delayed germination, slow growth
III	5.6	5.6	5.7	5.8	.221	6.2	.448	6.2	.678	Quick germination, excellent growth
IV	7.8	7.7	7.7	7.2	.077	6.2	.211	6.2	.445	Delayed germination
V	8.8	8.3	8.3	8.3	.0	8.4	.0	8.2	Trace	Few spores beginning growth
Control	5.6			5.5		5.7		5.7		
	4.2	4.2	4.2	4.2		4.2		4.1		Standard buffer solution

The limits for germination and growth were found to be pH 3.2, and pH 8.2, up to 15 days. The series which had initial pH values of 3.6 and 7.8 were about equally favorable up to 15 days, but, subsequently, the advantage was distinctly with the latter series. The spores germinated quickly at initial pH 5.6. At pH 3.6 and 7.8 this was slower, while a considerably higher percentage of the spores germinated at pH 3.6 than at initial pH 7.8, during the first seven days. These results agree with those of Webb (10), *viz.*, that increasing acidity favorably influences the germination of spores. The photograph in Plate II, Fig. 3, taken on the eighth day, illustrates the relative germination of the spores at pH 3.6 and 7.8. The original colonies at pH 5.6 were obscured by general growth within the decoction at this time.

Since after 20 days the pH value of media series IV was raised from 7.8 to 6.2, and the pH value of media series III was depressed from 5.6 to 6.2, it may be assumed that one growth optimum exists near a pH of 6.2. Moreover, the isoelectric point of the living fungus is between a pH 5.5 and 6.0. Referring to the work of Scott (9), another optimum for growth possibly exists between the isoelectric point and the higher pH value of 3.7, but this was not shown, because of the absence of series in this region.

If the growth of *P. meliloti*, as indicated in Table I and Text-fig. 2, is a fair indication of its growth in soils of corresponding pH values, it would appear that the cultivated horizon of typically black or wooded soils of Alberta and Saskatchewan, being commonly between pH 6 and 7, would be favorable to good growth of this fungus. Similarly, the brown soils, typical of the prairie belt, because of their alkaline reaction, commonly being from pH 7.0 to 8 or greater, would be only from slightly favorable to distinctly unfavorable. This observation is based on the work of Wyatt and Newton (12), who state that the pH values of the A_1 horizon of typically wooded soil, black soil, and brown soil, of Alberta, are 6.2, 6.0 and 7.2 to 8 or greater, respectively. Obviously, certain variations in reaction would occur within

each soil belt, according to soil. Data supplied by Mitchell (4), and by Saskatchewan Soil Survey Reports 1 to 9, indicate that, in general, the reaction of corresponding types of soil in that province is similar to those of Alberta.

Pathogenicity

Results of studies on the pathogenicity of *P. meliloti* are given in the following experiments:—

Experiment 1

During March, 1927, sweet clover plants were grown from disinfected seed, in sterilized soil, heavily inoculated with a vigorous culture of the fungus. The soil was kept at 12 to 13°, 17 to 18°, 20 to 21°, and 26 to 27° C., by the aid of temperature control tanks. There were 160 mature plants at each temperature, 40 of which were controls. When these plants were harvested five months later, the roots were apparently healthy. These results suggested that the fungus was not parasitic on normal plants at a temperature favorable to the growth of both plant and pathogen.

Experiment 2

The object here was to determine whether, at high or low soil temperatures, the cutting off of the tap roots of seedling sweet clover plants increases their susceptibility to brown root rot. The soil temperatures were 4 to 5° and 17 to 20° C. The tap roots of the seedling plants were from 2 to 3 mm. in diameter. The tap roots were cut off about two inches below the crowns. Sixty plants, the tap roots of which were cut off, were planted in the inoculated soil at each temperature. The control series for each temperature contained 20 normal plants, as well as 20 plants with tap roots cut off, all planted in sterilized soil.

Seventeen days later the roots were examined. In the inoculated soil, kept at 4° to 5° C., all the roots, whether cut or uncut, were unhealthy, with pycnidia of *P. meliloti* thereon. Likewise, the roots of the control series had many dead areas, and were shrunken and apparently nearly dead but without pycnidia. The roots of all plants at 17 to 20° C. were growing well and were apparently very healthy.

Experiment 3

This was a repetition of Experiment 2, except that the tap roots were not cut off, and that the low soil temperature was approximately three degrees higher, *viz.*, 5 to 8° C. Vigorous plants, from the same source as those used for Experiment 2, were transplanted into the inoculated and uninoculated soils, at the conclusion of Experiment 2, that is, 17 days after that experiment was begun.

The roots in both temperature series were examined at the end of 48 days. A total of 108 dead areas developed on the roots of the 60 plants in the inoculated soil, while 29 similar areas appeared on the 30 control plants in the uninoculated soil. Very few new roots started to develop at this temper-

ature, and those which the plants had, when transplanted, lost their vigor. At 17 to 20° C. all of the plants grew well, the roots being perfectly healthy.

The results of the foregoing experiments indicated that unfrozen roots of sweet clover, up to four months of age, were immune to *P. meliloti* at soil temperatures 12 to 27° C. Whether such roots were also immune at a temperature range of 4 to 8° C. could not be determined from the experiments made, since the roots of the plants used failed to produce new roots, and also tended to become senile at temperatures from 4 to 8° C.

Experiment 4

The object here was to determine whether first-year roots of sweet clover and alfalfa, growing in the field, were immune to *P. meliloti* prior to winter dormancy. Seed of *Melilotus alba* (varieties—Arctic, Common White and Zouave), and of *Medicago sativa* (varieties—Grimm and Baltic), known to be susceptible, was sown June 15. The roots of the plants were inoculated as they grew in the field August 7. Each test comprised ten roots, inoculated, and an equal number of control roots, located alternately in the row with the inoculated ones. Both test and control roots were treated similarly, except that the latter did not receive inoculum. The fungus was applied to the tissues in three ways: (a), in a cavity made by passing a small cork-borer through the tap root, about two inches below the crown; (b), against the uninjured tap root, about two inches below the crown; and (c), against the cut off ends, and also against the normal ends of lateral roots. In all cases the root surface to be treated was washed with mercuric chloride solution, followed by sterilized water. The inoculum, grown on ground oat hulls, was placed in position and protected by a wrapping of cotton. Finally, the excavated soil was returned about the roots and firmed.

When the roots were removed from the freezing soil at the beginning of November, the treated and control roots, alike, were sound. The cavities which had been made were calloused, and the tap roots had grown well, being from 15 to 20 mm. in diameter. It was concluded, from this and previous experiments, that first-year roots of sweet clover and alfalfa are not susceptible to *P. meliloti* before winter dormancy.

Experiment 5

This was to determine the susceptibility of roots of sweet clover, alfalfa, and common clover to *P. meliloti* during the winter dormancy period. Five varieties of sweet clover, four of alfalfa, and nine of common clover were tested, all of which are named later in the section of this paper entitled "hosts". The three methods of applying inoculum to the roots, mentioned in the foregoing experiment, were used on each of the varieties employed in this test. The use and arrangement of control roots, the preparation of roots for inoculation, and subsequent care, were the same as described in Experiment 4. The roots were treated as the ground froze during early November, and they were examined early next May.

The results of this experiment, with regard to sweet clover and alfalfa, were so definite, and for each so uniform, that they may be given briefly. Every inoculation, regardless of method used, or variety, was successful in producing the lesion characteristic of brown root rot. In general, where the inoculum had been placed in cavities in the tap root, the lesions were more severe than where it had been placed against the uninjured tap root, although in many cases of the latter, maximum injury occurred. The inoculations on the cut or uncut lateral roots were equally effective (Plate II, Fig. 5, *A*), and, in many cases the decay entered the tap root. In Plate II, Fig. 5, *B* and *C*, are photographs of typical lesions of brown root rot, experimentally produced by contact inoculation on sweet clover and alfalfa, respectively.

To indicate the growth of *P. meliloti* during the winter, several slants of potato dextrose agar were inoculated and immediately placed about 4 in. deep in the frozen soil, near the roots. The photograph in Plate II, Fig. 4, illustrates the excellent vegetative growth and abundance of pycnidia, which developed from November until April 15, following.

The roots of common clover also proved to be susceptible to *P. meliloti*, but the degree of this was much obscured by severe winter injury which many of the varieties suffered. However, typical lesions, with pycnidia, were produced experimentally on the two hardier varieties, Late Swedish Red, and Altaswede. On all inoculated roots of other varieties, dead or suffering from ordinary winter injury, there were numerous pycnidia of *P. meliloti*. Probably this fungus was associated chiefly as a saprophyte in the latter instances, but there is still the possibility that it had increased the injury primarily arising from lack of winter hardiness. This phase of the problem is being investigated.

Experiment 6

Since *P. meliloti* penetrated easily and rotted the roots of sweet clover emerging from the dormancy stage (Experiment 5), it was important to determine the effect of soil temperature on the development of the disease. Accordingly, large plants of *M. alba*, from seed planted the previous June, were removed from the frozen field soil March 1. The surface of these roots was treated with mercuric chloride solution, then washed, and inoculated with the pathogen, and planted immediately in sterilized soil of optimum moisture. The inoculum was placed in a cavity in the tap root, as described earlier, and protected by a wrapping of sterilized cotton. The controls were prepared similarly, but without the pathogen. Series, consisting of 30 inoculated roots, and 10 control roots, were kept at 2–3°, 9° and 16° C., respectively. The roots were examined for disease at the end of 21 days.

At 2–3° C., 1 root had a trace of rot; 4 roots were decayed slightly; 8 had light lesions; 12, medium decay, and 4 roots had severe lesions. At 9° C., 3 roots had slight lesions; 5 had light lesions; 13, medium, and 9 roots had severe lesions. Of the roots at 16° C., 2 had slight lesions; 7 light, 12 medium, and 9 severe lesions. The controls were sound. The growth of the crown foliage

on the roots at 16° was good, that at 9° fair, while only slight growth occurred at 2–3° C. If the roots at the beginning of the experiment were about equally susceptible, the development of the disease would depend partly on the growth of the fungus, and partly on the time taken for recovery of the host from dormancy, both of which would be favored by a temperature of 16° C. At 16° and 9° C. the disease developed about equally well, while at 2–3° C. it was retarded. However, if the test had run longer than 21 days the final results might have been reversed.

Experiment 7

This was to determine the progress of brown root rot at temperatures 2–3°, 9° and 16° C. in normal roots of sweet clover, artificially frozen and not frozen. The roots used were from sweet clover plants about three months old, and grown in the greenhouse. These were inoculated by the core method, described in Experiment 5, and planted in sterilized soil of optimum moisture. There were 20 inoculated roots, and 10 control roots in each frozen and unfrozen series of the temperatures mentioned. The roots for the frozen series were first hardened off at 5°, 0°, and −4°, then to 3° C., each change being for four days. The plants of each of the two series mentioned were held at 2–3°, 9°, and about 16° C., respectively, for 21 days, when they were examined for disease.

The results of this experiment were that both inoculated and control roots remained sound at all temperatures, in both frozen and unfrozen series. New root growth at 3° C. on both series was negligible. There was some evidence of senility of the older rootlets. At 9°, new roots developed fairly well, but slowly, while at 16° C. new roots were abundant. Excellent top growth occurred at 16°, it was fair at 9°, and very slow at 2–3° C. Thus normal, unfrozen roots of sweet clover appear to be immune to *P. meliloti* at temperatures above 2° C. Also, the freezing of them at −4° C. for four days does not make them susceptible.

Hosts

Although field tests of the relative resistance of varieties of alfalfa, common clover and sweet clover to *P. meliloti* are still incomplete, experimental evidence to date indicates that sweet clover is more susceptible than either common clover or alfalfa. The following varieties of sweet clover, alfalfa, and common clover were found to be susceptible by tests made in the field: *Sweet Clover*,—Arctic, Common White, Maccor, Zouave, Grundy; *Alfalfa*,—Grimm, Baltic, Cherno, Cossack; *Common Clover*,—Late Swedish Red, Altaswede, St. Clett, Trystofte, Dauphene, Mammoth, Albert, Kenora, Spadone.

Robertson (6) found that a culture of *P. meliloti*, isolated from hollyhock (*Althaea rosea*), was pathogenic to roots of this plant, and also to roots of sweet clover, during winter dormancy.

In addition to the hosts mentioned, pycnidia were found during May on dead roots of *Axyris amaranthoides*, *Amaranthus retroflexus* and *Avena sativa*,

the relationship being strictly saprophytic. Pycnidia were abundant on the first host, very scarce on the second, and only one body was found on oats. Also, no bodies were found on the roots of dead wheat stubble, whereas, on sweet clover, in the same plot, pycnidia were very abundant.

Persistence in Dry Soils

To determine how long this pathogen might survive in dry soils, a loosely plugged 200-cc. flask of sterilized soil was inoculated with a spore suspension of *P. meliloti*, and incubated at about 18° C. for 10 days, until a substantial growth of mycelium had ramified the soil. The culture was then kept at room temperature for two years, during which time fragments of the soil were cultured in Petri plates on potato dextrose agar. The moisture content of the soil at the end of 60 days was 9.3% of its moisture-holding capacity, and at the end of 20 months, 4·4%. The fungus grew from the dry soil up to 20 months, but not longer. Microscopic examination of the soil showed that the fungus persisted in the form of small sclerotia-like masses, which probably were immature pycnidia, since *P. meliloti* has not been observed to produce real sclerotia, either in culture or in the tissue of the host. These results suggest that pycnidia might remain viable in unusually dry soils, at least one year.

Occurrence in Virgin Soils

As *P. meliloti* was so common in recently virgin soils, it seemed probable it was native to strictly virgin prairie sod. Accordingly, the crown portion was removed from 50 vigorous tap roots of *M. alba*, to prevent growth, and the roots disinfected and washed. These were distributed late in October in the black loam virgin prairie sod at Edmonton, and left buried until May, the following spring, at which time the roots were more or less decayed. Several of these roots bore pycnidia from which *P. meliloti* was isolated.

Control

The danger of contaminating new soil, in an area already infested, by means of spores of the pathogen carried by sweet clover seed or foliage, would seem unimportant in practice. Probably the wind is the most effective agent in carrying this fungus from one field to another. Control of brown root rot by crop rotation, or by a naturally resistant variety, seem the only methods available. However, the writer's studies in connection with either method have not yet yielded definite information. Seeding sweet clover in soil where brown root rot has been prevalent would be unsafe, unless preceded by immune crops.

From observation and experiment, brown root rot appears to progress as well in large plants as in small ones. On the other hand, the large roots seem to survive more often than small roots, because small tap roots, with a limited lateral system, are the more easily killed. Thus, large, vigorous plants should be produced the first season, and seeding without a nurse crop is, perhaps, the best way to do this.

Discussion

The foregoing results show that, under field or laboratory conditions, the roots of sweet clover, common clover and alfalfa are not susceptible to *P. meliloti* prior to the winter dormancy stage, but that they become very susceptible during late winter and early spring. The changed biological condition of the internal tissue of the roots, resulting from the long dormant condition, seems to offer the best explanation for this. Whether the marked susceptibility can be attributed to an increase of food materials favorable to the fungus at this time, or to the failure of the cells of the partially dormant tissue to function normally in resisting penetration, are interesting questions. In this connection it was observed that the roots of sweet clover and alfalfa commonly are more or less shrunken by late winter, and that the cells of the root tissue apparently lack turgor. However, when new crown growth starts and new roots are proliferated on the advent of higher soil temperatures, the roots soon return to normal plumpness and rigidity, characteristic of summer growth. Coincident with this return to normalcy is the marked slowing up and final arrest of the disease.

Jones (3) found that factors of climate and soil during winter caused certain internal and external mechanical injuries to the roots of alfalfa. Weimer (11) produced a number of these injuries, including the killing of the tender root tips, by artificial freezing. Macroscopic evidence during these studies indicated that similar external injuries occur on the root tips of sweet clover during winter in western Canada, and that such areas greatly favor the incidence of brown root rot. On the other hand, there were numerous cases where *P. meliloti* entered the root surface in the absence of rootlets or mechanical injuries.

With regard to the effect of low soil temperatures on brown root rot of sweet clover, while in a dormant or semidormant condition, evidence during the last four years has been that inoculated roots, at Edmonton and Lacombe, have suffered as severely in one year as in another, while uninoculated plants have remained sound. Thus, true winter-killing and brown root rot are distinct.

Finally, if climatic and other factors, during winter and early spring, alter the complete resistance of the roots to a condition of marked susceptibility to *P. meliloti*, various degrees of this might be expected among varieties and roots within a variety, depending upon the reaction of their individual physiology to the environment. Similarly, in the case of true winter-killing, there would be various degrees of physiologic modification. Therefore, it seems probable that other soil-inhabiting fungi and bacteria, under certain conditions, contribute to the mortality arising from lack of winter hardiness, as does *P. meliloti*.

Acknowledgments

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THE RELATION OF SPECIES OF AGROPYRON AND CERTAIN OTHER GRASSES TO THE FOOT-ROT PROBLEM OF WHEAT IN ALBERTA[1]

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Abstract

A survey was made of certain wild and cultivated grasses in Alberta in order to ascertain to what extent they are harboring fungi capable of causing foot rots of wheat. Quack grass, *Agropyron repens*, and western rye grass, *Agropyron tenerum*, were found to be particularly important in this respect. Both were found heavily attacked under natural conditions with strains of the take-all fungus, *Ophiobolus graminis*, which proved capable of causing as severe damage to wheat as strains from wheat. Strains of *Helminthosporium sativum* which proved highly pathogenic to wheat were also isolated from these two grasses. Strains of *Fusarium* obtained from *A. repens* and *A. richardsonii* caused little or no damage to wheat.

Agropyron repens is already an important weed in central Alberta. It was found infesting cultivated fields in summerfallow as well as those in crop. In summerfallow this weed appears to encourage the survival of *Ophiobolus graminis*, while in wheat fields infected quack grass was found associated with severe take-all damage to the crop. *Agropyron tenerum* is one of our most popular forage grasses and occurs commonly as a wild native plant in Alberta. Observations indicate that in the moister parts of the province wheat following this grass in rotations may be severely injured by take-all. In a rotation at the University of Alberta, wheat showed little or no take-all damage after timothy and alfalfa, moderate damage after brome grass and severe damage after western rye grass. In this experiment western rye grass itself was almost killed out prematurely in all replicates, apparently by the take-all fungus.

Artificial inoculation of the various grasses was made with wheat strains of foot-rotting fungi by adding inoculum to the soil. All species of *Agropyron* tested including crested wheat grass, *Agropyron cristatum*, proved highly susceptible to *Ophiobolus graminis*, moderately susceptible to *Helminthosporium sativum*, but only slightly susceptible to *Fusarium graminearum*, though the latter was responsible for considerable non-emergence of the seedlings. *Bromus inermis* and *B. ciliatus* proved quite susceptible to all three pathogenes. *Hordeum jubatum* was heavily attacked by *Ophiobolus graminis* but not by the other two fungi. *Avena sativa* was not attacked by *O. graminis* and only slightly by *H. sativum* and *F. graminearum*, while timothy, *Phleum pratense*, appeared immune from all three fungi.

Introduction

Numerous reports have been made of fungi causing foot rots of wheat on wild and cultivated grasses including members of the genus *Agropyron*. The purpose of the present investigation was to determine as far as possible the extent to which some of these grasses serve in increasing the damage to wheat caused by foot rots and to find whether the various grasses common in Alberta exhibit any appreciable differences in the extent to which they intensify the problem.

Early workers reporting the production of perithecia of *Ophiobolus graminis*, the organism causing take-all of wheat, on species of *Agropyron* were Saccardo in 1875, Waters (13) in 1920, and Brittlebank (1) in 1919. Waters reported production of perithecia on *A. repens* and Brittlebank on *A. scabra*.

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Kirby (5) in 1921 found *A. repens* commonly affected with take-all in New York State under natural conditions. In addition it was found that all six species of *Agropyron* studied, namely, *A. caninum*, *A. cristatum*, *A. intermedium*, *A. repens*, *A. smithii* and *A. tenerum* became infected when seeded in pots of clean unsterilized soil which had inoculum added in the form of pure fungous mycelium grown on steamed wheat kernels. Perithecia were produced by all six species in varying numbers, the writer stating that, "since the important role of the grasses would be as carriers of the disease the chief object of this test was the determination of the grasses on which perithecia were produced". The same writer (6) in 1925 conducted a still more extensive experiment, this time using sterilized soil. Together with many other grasses, 11 species of *Agropyron* were tested, namely, *A. caninum*, *A. cristatum*, *A. desertorum*, *A. intermedium*, *A. obtusiusculum*, *A. repens*, *A. richardsonii*, *A. scabrum*, *A. smithii*, *A. spicatum* and *A. tenerum*, on all of which perithecia were developed. The only occurrence in the field, however, was in the case of *A. repens*. The writer again states that the importance of any grass as a harborer of the pathogene causing take-all of wheat depends on the number of perithecia produced.

Russell (11) believes that brome grass (*Bromus inermis*), western rye grass (*A. tenerum*) and quack grass (*A. repens*) all serve to increase the amount of take-all in crops of wheat following them in the rotation.

Bolley in 1911 found a species of *Helminthosporium* causing infection of *A. repens* in North Dakota and Wisconsin, and apparently regarded it as identical with that causing foot rot of wheat. Dreschler (4) found *H. sativum* commonly caused a leaf-spot disease of *A. repens* in the midwestern states of the United States. Other investigators report successful inoculation of hosts in several different genera with *H. sativum*. Stakman (12) in 1920 inoculated the leaves of a number of grasses with strains of *H. sativum* obtained from wheat and rye, and successfully infected those of *Agropyron smithii*, *A. repens*, and *Hordeum jubatum*. No leaf lesions appeared on *Phleum pratense*. The root systems were not inoculated. Christensen (2) succeeded in isolating a species of *Helminthosporium* which he believed to be *H. sativum* from aboveground parts of *A. caninum*, *A. desertorum*, *A. repens*, *A. smithii* and *A. tenerum*, as well as from a number of other grasses under natural conditions in various parts of the United States. In pots of sterilized soil in the greenhouse the aboveground parts of seven species of *Agropyron* inoculated with *H. sativum* all showed infection to a more or less marked degree. Heavy infection occurred on *A. caninum*, *A. spicatum* and *A. tenerum, longifolium*; *A. tenerum* and *A. repens* showed medium infection, and only light infection was apparent on *A. cristatum*, *A. desertorum* and *A. smithii*. In these cases root infections were not studied especially, but a small additional experiment showed the roots of *A. tenerum* to be infected. Attention is called to several possibilities in relation to these and other susceptible grasses; that they may serve to increase secondary infection of cereals; that they may aid in overwintering; and that windblown spores from foliages of susceptible grasses may cause

infection of wheat on comparatively clean soil. These workers did not especially direct their attention to the importance of these grasses in connection with the foot-rot problem, but instead restricted their studies largely to the leaf-spot diseases.

Several workers have reported the occurrence of *Giberella saubinetlii*, the perfect stage of *Fusarium graminearum*, under natural conditions on *Agropyron smithii*, *A. repens*, *A. tenerum* and *A. caninum* causing head blights, and the list was extended by MacInnes and Fogelman (8) at Minnesota in 1923 to include under experimental conditions a number of grasses, amongst them *Agropyron desertorum*, *Bromus inermis* and *Phleum pratense*.

Distribution of Grasses in Alberta

Ample literature is available concerning the distribution of the more important grasses in Alberta. Many of the most important species in relation to the foot-rot problem of wheat occur in the genus *Agropyron*, which has been chosen mainly for this investigation. However, several other genera contain native or introduced species with a wide distribution in Alberta, and the more common of these are included, namely, *Bromus inermis*, *B. ciliatus*, *Hordeum jubatum*, *Avena fatua* and *Phleum pratense*. The distribution of these grasses in Alberta will be described briefly, and for more detailed information the investigations of Clarke, Moss, Peto and Preston may be consulted (3, 7, 9, 10).

Agropyron tenerum and *A. richardsonii*

These grasses are distributed throughout a large part of the province of Alberta, occurring most commonly in the northern and parkland belts. Western rye grass (*Agropyron tenerum*) is widely cultivated in the province.

Agropyron repens

This weed has become common in many parts of Alberta, especially in the central part, its persistence being due largely to its numerous creeping rootstocks. Quack grass is prevalent in the Edmonton district, and during the summer of 1932 the writers observed few summerfallow fields in this district completely free from the weed. In some fields of well-worked fallow where all other weeds were kept well under control, quite thick stands of quack grass were noticeable in patches throughout the fields. At least three fields of almost pure quack grass were noticed which were being used for hay.

Agropyron smithii

This is a common constituent of the short grass plains, occurs to a considerable extent in the transition belt or parkland areas, and is found in central and northern Alberta as a rule only on drier and more exposed slopes. It usually does not encroach seriously upon cultivated fields except in very dry areas.

Agropyron caninum

This species has been reported to occur amongst the upland grasses of the Grande Prairie-Beaverlodge district of northern Alberta. It is not a common grass in central and southern Alberta.

Agropyron griffithsii

A. griffithsii occurs mainly on the dark brown soils of the prairie. It does not as a rule form a thick sod and seems to occur mainly as a few scattered plants within a district. It rarely occurs in cultivated fields.

Agropyron cristatum

This grass (known under the common name of crested wheat grass), was introduced from European Russia, and is recommended for cultivation in districts of limited rainfall. The grass spreads to some extent by underground stems, but does not produce long creeping rhizomes like those found n *A. repens*. The likelihood of more extensive use of this grass as a cultivated orage crop in the future is to be considered.

Agropyron dasystachyum

This grass resembles *A. smithii* in its distribution, being found mainly in the southern part of the province, but is less tolerant towards excessive moisture and is therefore fairly well restricted to the southern and drier parts of the province.

Agropyron sibiricum, *A. obtusiusculum*, *A. desertorum* and *A. elongatum*

For the most part natives of Russia, these grasses have been introduced mainly for experimental purposes. In view of the possible development of some of these species they were studied to some extent in connection with the host range of the foot-rotting organisms concerned.

Bromus inermis

Awnless brome grass is grown to a large extent in Alberta as a hay and pasture crop, especially in central Alberta. It is quite commonly grown on sandy land, but is distributed over all types of soil. Throughout central Alberta it has escaped from cultivation, often growing thickly along roadsides. It is a moderately difficult grass to eradicate.

Bromus ciliatus

This is a native brome grass found commonly in northern and central Alberta but as a rule not forming a thick sod. It is found quite commonly on sandy lands and hillsides. It is not a serious weed.

Hordeum jubatum

H. jubatum is a perennial grass, distributed throughout Alberta, and seems equally suited to dry sandy soils and moist bottom lands. It occurs commonly in cultivated fields and spreads rapidly by seed; hay crops are frequently badly contaminated with this weed. It is prevalent on waste land.

Avena fatua

It is hardly necessary to say more than that this weed is generally distributed throughout Alberta and is very commonly found in cultivated crops.

Phleum pratense

Timothy is cultivated in some of the moister districts, and is found to a small extent escaped from cultivation.

Occurrence of Foot-rotting Organisms of Wheat on Grasses Under Natural Conditions

During the summer of 1932 a brief survey was made of several districts of Alberta with a view to determining the frequency of occurrence of wheat foot-rotting organisms on grasses. This work was done in central Alberta, automobile trips being made from Edmonton, south to Innisfail, east to Provost and Lloydminster on the Saskatchewan border, and north from Edmonton to Clyde and Westlock. Notes made on a few of the fields examined, together with some observations made in the Edmonton district at previous dates, are summarized below according to the species of host.

Agropyron repens

One of the first isolations made was that of *Helminthosporium sativum* from *A. repens* at Edmonton in September, 1931.

At Edmonton in June 1932, a field was examined which had been left uncultivated for several years and had become infested with quack grass, which was found to be stunted in large circular patches. *Ophiobolus graminis* was isolated without difficulty from rhizomes in these patches. The blackened appearance of these rhizomes is well demonstrated in Fig. 1. The strain was numbered 104.

FIG. 1. *Left: Rhizomes of A. repens from a stunted patch affected with O. graminis. Right: Healthy rhizomes from just outside the patch.*

A field of *A. repens* of about 10 acres was found at Blackfoot, August 25. The field had been cut for hay and the quack grass was stretching far into adjacent fields of wheat and barley. The rhizomes were heavily affected with take-all. The wheat in the infested field was also severely damaged by take-all, the damage being estimated at 30% and the degree of infection at 51%.

Quack grass was found to be a common weed in a 100-acre field of western rye grass at Vermilion, August 25. There was severe infection of the rhizomes and *Helminthosporium satwum* and a *Colletotrichum* sp. were isolated.

A 20-acre field of stubble was examined at Vegreville on August 25. It had apparently been intended for summerfallow but had not been worked up to this date. Considerable quack grass was present, heavily affected with take-all caused by *O. graminis*.

At Josephburg, August 26, a summerfallow field was noticed in which the distribution of quack grass was general. Numerous lesions appeared on the rhizomes and *O. graminis* was identified as the causal organism.

Heavy infestation of summerfallow with *A. repens* was observed at Fort Saskatchewan, August 26. The rhizomes showed symptoms typical of those caused by *O. graminis*, though the fungus was not isolated.

FIG. 2. *Right foreground: A western rye grass plot, showing areas where A. tenerum plants have been killed and a number of encroaching grasses and weeds. Left foreground: timothy. Right background: alfalfa. Left background: brome.*

A summerfallow field of 300 acres examined at Fort Saskatchewan on August 26 was infested with *A. repens* heavily infected with *O. graminis*. A *Fusarium* sp. was also isolated.

A wheat field of about 50 acres at Oliver was found heavily infested with quack grass resulting in pronounced stunting of the wheat. The quack grass was very heavily infected with *O. graminis*, and the wheat showed as much as 10% damage and 30% infection in patches.

Only two cases were found in which the rhizomes of *A. repens* were free from lesions. *O. graminis* was suspected in many cases other than those reported.

Agropyron tenerum

Experimental plots of western rye grass at the University of Alberta were found in May 1932 to be dying out. There is evidence that this was due to a large extent to foot rot. *O. graminis* was isolated from many of the roots. *H. sativum* was also isolated. The history of the plots is described later, but the severe damage and subsequent invasion by weeds can be seen from the photograph (Fig. 2).

A field of about 100 acres at Vermilion on August 25 appeared to be suffering severely from foot rot. *H. sativum* was isolated. The symptoms of take-all appeared to be present, but attempts to isolate *O. graminis* were unsuccessful.

Species of *Fusarium* and an organism resembling a *Leptosphaeria* sp. were isolated in several instances from *A. tenerum.*

Bromus inermis

Foot rot was found in only six out of twenty-three fields of *B. inermis* examined, and in no instance did the damage appear severe. *Helminthosporium sativum* and a *Fusarium* sp. were isolated, but *Ophiobolus graminis* was not found on this grass even when it was growing amongst infected plants of *A. repens.* It is possible, however, that the organism was present on the fine roots of the grass.

Foot rots were also observed on *Agropyron richardsonii* and *A. smithii,* and *H. sativum* was isolated from the former.

In all, only 29% of the fields of *Bromus inermis* examined appeared to be affected with foot rot, while 64.3% of the *Agropyron tenerum* fields and almost all the *A. repens* fields were affected.

Pathogenicity of *Ophiobolus graminis, Helminthosporium sativum,* and *Fusarium* spp.

It is of importance to know whether those strains of foot-rotting organisms found on grasses are capable of causing damage to wheat. For this reason several strains of each fungus isolated from grasses were compared with strains of similar organisms obtained from wheat. The tests with *H. sativum* strains were conducted in October 1931; the tests with *O. graminis* and *Fusarium* strains were conducted almost a year later but the methods adopted were similar. The strains used are described below.

Ophiobolus graminis

Strain 103. From wheat grown on soil from plots of *A. tenerum* at the University of Alberta.

Strain 108. Obtained from *A. repens* at Oliver, Alberta.

Strain 104. From rhizomes of *A. repens* near Edmonton, Alberta.

Strain 101. Obtained from *A. tenerum* at the University of Alberta.

Helminthosporium sativum

Strain 6. Obtained from wheat at Brooks, Alberta. This was originally a monospore culture.

Strain 102. From *A. repens* rhizomes at Edmonton, Alberta.

Strain 101. Obtained from the bases of stems of *A. tenerum* on plots at the University of Alberta.

Fusarium Strains

Strain 2. *Fusarium graminearum* from wheat at New Norway, Alberta.

Strain 101. From wheat at Peace River, Alberta.

Strain 102. From *A. repens* at Oliver, Alberta.

Strain 103. Isolated from *A. richardsonii* at Oliver, Alberta.

Soil cultures of the different isolations were grown in Erlenmeyer flasks, eight replicates of each being used in the *H. sativum* tests, but only three replicates of each strain of *Fusarium* and *O. graminis*. For the *Ophiobolus* cultures 10% of cornmeal was added to the soil. To each Erlenmeyer flask 50 gm. of soil (or soil and cornmeal) was added, and 28 cc. of tap water. The flasks were placed in the autoclave and the contents sterilized at 15 lb. pressure for one hour, after which the cultures were started by introducing the fungi. After seventeen days' growth the contents of each flask was added to a pot of sterilized soil, and each pot was seeded with 25 seeds of Marquis wheat and placed in the greenhouse. After nearly three weeks' growth the pots were harvested and the degree of infection and length of the plants were measured. The results are summarized in Tables I, II and III.

TABLE I

PATHOGENICITY OF STRAINS OF *Ophiobolus graminis* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
103 From wheat	82.7	10.1	72.9
108 From *A. repens*	68.0	8.8	87.4
101 From *A. tenerum*	80.0	9.9	74.0
104 From *A. repens*	70.7	8.6	83.0
Check—no organism	76.0	19.3	1.4

TABLE II

PATHOGENICITY OF STRAINS OF *Helminthosporium sativum* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
6 From wheat	47.0	10.7	51.9
101 From *A. tenerum*	43.0	11.3	41.7
102 From *A. repens*	57.0	9.4	55.8
Check—no organism	87.5	16.0	1.0

TABLE III

PATHOGENICITY OF STRAINS OF *Fusarium* ON MARQUIS WHEAT

Strain of organism, and origin	Emergence of wheat plants, %	Average length of plants, cm.	Average degree of infection, %
2 From wheat	9.3	6.7	80.0
101 From wheat	61·3	14·9	0.0
102 From *A. repens*	66.0	14.7	6.0
103 From *A. richardsonii*	57.3	17.2	0.0
Check—no organism	76.0	19.3	1.4

It is seen that strains of *O. graminis* and *H. sativum* isolated from grasses are generally as pathogenic as are the strains isolated directly from wheat itself. There appear to be slight differences in pathogenicity between the different strains of each of these organisms, and for *Helminthosporium* it was found that these differences were possibly significant in the light of the probable errors, which were calculated but are not included in the tables. These differences were not considered to be of practical importance. The fact that strains of these two organisms obtained from species of *Agropyron* growing under natural conditions are very pathogenic to wheat, however, is of considerable practical significance. The *Fusarium* strains obtained from grasses, with the possible exception of that obtained from *A. repens* were not pathogenic on wheat under the conditions of the experiment, but other experiments (since completed) go to show that these grasses may nevertheless under some conditions harbor forms of *Fusarium* pathogenic towards wheat. The common occurrence of the latter condition in nature is not yet proved.

The Host Range of *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P.K.B. and *Fusarium graminearum* Schwabe

Previous work on the host range of *O. graminis* has been confined mainly to determination of the host species on which the fungus produces perithecia, while with the other two organisms the work has been confined mainly to the aboveground parts of the hosts.

While such methods of determining the reaction of the various grasses may be useful for certain purposes, it seemed that in relation to the foot-rot problem of wheat it was more important to determine the reaction of the basal or underground parts of the grasses to soil-borne inoculum. It was considered that such determinations would also give a better index of the relation which the various grasses might have to the infestation of the soil. Consequently all of the data reported are based on the reaction to soil-borne inoculum, prepared and used as described below.

The strains of fungi used in the study were: *O. graminis*, 108; *H. sativum*, 6; and *F. graminearum*, 2. Litre flasks containing 200 gm. each of soil with 10% cornmeal added were moistened with 112 cc. each of tap water and sterilized.

The organisms were then added, using six flasks for *Fusarium* and twelve each for *Ophiobolus* and *Helminthosporium*. This inoculum was allowed to grow for 17 days at room temperature. Pots of sterilized soil, to which were added separately the three different organisms, were seeded with the various species of grasses. A duplicate series was planted. In addition a similar series was prepared in which unsterilized soil was used. The inoculum was then added as evenly as possible to infest the soil as required and the pots were then seeded to approximately equal numbers of seeds of the various grasses. They were placed in the greenhouse at an air temperature of approximately 70° F. and were grown for 52 days. The pots were photographed (Fig. 3), and the plants harvested, measured and scored for infection. Roots from the *H. sativum* and *F. graminearum* series were surface sterilized with silver nitrate and plated out on potato dextrose agar where infection was suspected; plants and roots of the *Ophiobolus* series were examined with a microscope. The combined results are found in Tables IV and V.

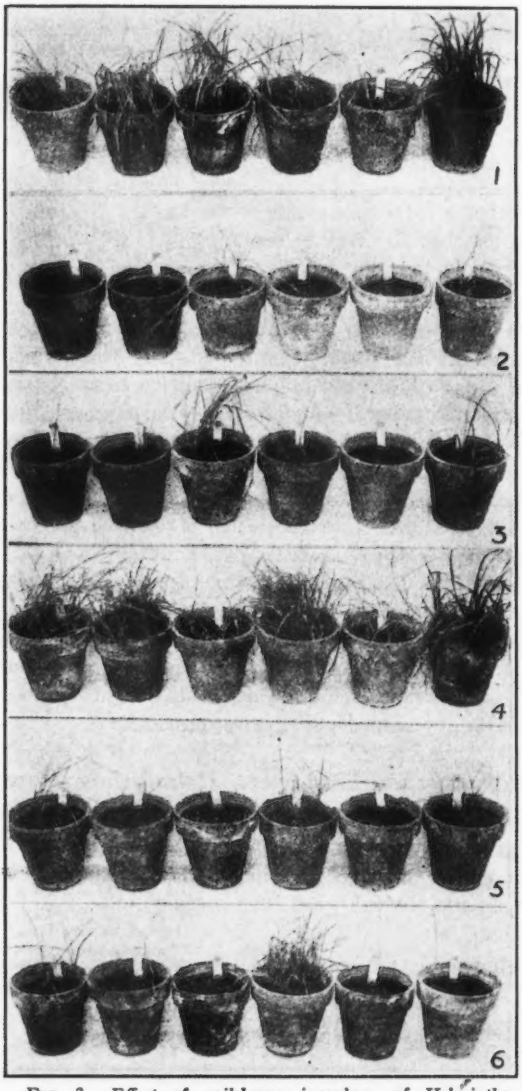

FIG. 3. *Effect of soil-borne inoculum of Helminthosporium sativum and Fusarium graminearum on Agropyron spp. Left to right (rows 1–3): A. cristatum, A. sibiricum, A. obtusiusculum, A. smithii, A. desertorum, A. caninum. Row 1, check (no organism added to soil); row 2, soil infested with H. sativum; row 3, soil infested with F. graminearum. Left to right (rows 4–6): A. elongatum, A. richardsonii, A. griffithsii, A. tenerum, A. dasystachyum, A. repens. Row 4, check (no organism added to soil); row 5, soil infested with H. sativum; row 6, soil infested with F. graminearum.*

While in this host range study special attention was given to the genus *Agropyron*, several species of grasses occurring as weeds or as crop plants in Alberta and belonging to other genera were included. Outside the genus *Agropyron*, the grasses studied were *Bromus inermis*, *B. ciliatus*, *Hordeum jubatum*, *Phleum pratense* and *Avena fatua*.

Agropyron elongatum was not tested with *O. graminis*. All other tested species of this genus* were susceptible when grown on both sterilized and unsterilized soils. *Bromus inermis*, *B. ciliatus* and *Hordeum jubatum* were all infected, but *Avena fatua* and *Phleum pratense* appeared not to be susceptible (see Fig. 4).

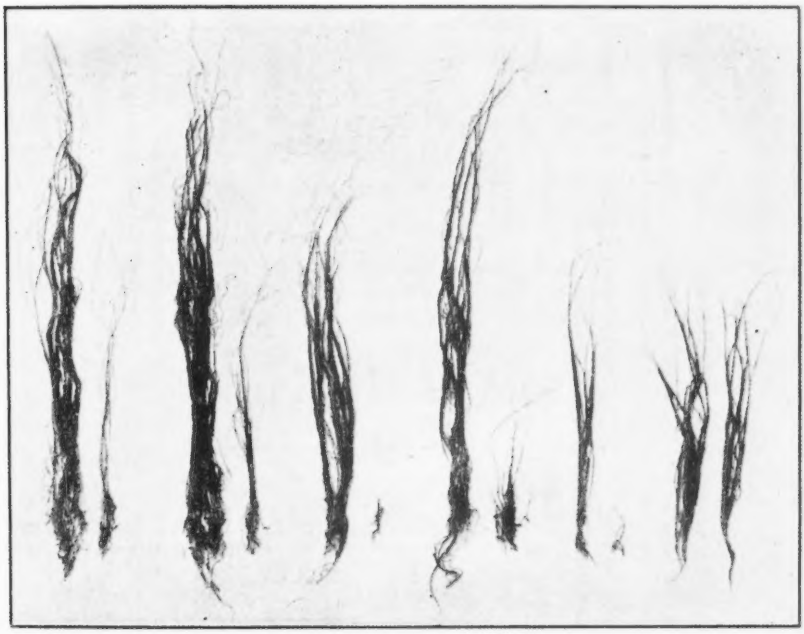

FIG. 4. *Effect of O. graminis on some grasses of economic importance. Check plants at left and plants from infested soil at right. Left to right: Agropyron tenerum, A. repens, A. cristatum, Bromus inermis, Hordeum jubatum and Phleum pratense.*

In the genus *Agropyron* all species were susceptible to *H. sativum*. The only cases where the organism was not isolated were with *A. caninum* on sterilized soil and with several species (*A. cristatum*, *A. desertorum*, *A. elongatum* and *A. griffithsii*) where plants were completely killed and the tissues had become so invaded by saprophytic fungi as to inhibit growth of *H. sativum* when plated on potato dextrose agar. *Bromus inermis* and *B. ciliatus* were both attacked though not severely injured and *Avena fatua* was infected lightly on sterilized soil. *Hordeum jubatum* and *Phleum pratense* were not infected (see Fig. 3).

*The writers are indebted to Dr. J. R. Fryer for supplying seed of these grasses.

TABLE IV

REACTION OF CERTAIN GRASSES TO *O. graminis*, *H. sativum* AND *F. graminearum* IN STERILIZED SOIL

Organism	*Ophiobolus graminis*			*Helminthosporium sativum*			*Fusarium graminearum*			Check—no organism	
Host species	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %
A. cristatum	2.0	100.0	+	No plants	....	....	19.2	0.0	−	21.1	0.0
A. sibiricum	2.7	100.0	+	22.5	60.0	+	17.8	0.0	−	25.8	0.0
A. obtusiusculum	4.9	100.0	+	16.4	21.8	+	30.7	0.0	−	33.2	0.0
A. smithii	No plants	....	....	10.8	40.0	+	15.0	0.0	−	13.8	0.0
A. desertorum	3.5	100.0	+	No plants	....	....	No plants	....	....	27.7	0.0
A. caninum	7.0	77.6	+	13.8	29.7	−	19.2	0.0	−	21.2	0.0
A. elongatum	Not seeded	....	....	21.2	21.4	+	28.6	4.0	+	26.6	0.0
A. richardsonii	9.3	93.2	+	13.4	40.0	+	14.0	0.0	−	15.8	0.0
A. griffithsii	3.8	98.4	+	No plants	....	....	No plants	....	....	15.1	0.0
A. tenerum	7.1	88.2	+	14.2	23.1	+	19.2	0.0	−	21.5	0.0
A. dasystachyum	5.8	86.7	+	12.7	40.0	+	No plants	....	....	18.5	0.0
A. repens	10.4	67.9	+	15.5	36.0	+	24.0	5.0	+	24.2	0.0
Bromus inermis	4.6	95.2	+	18.8	41.3	+	20.5	16.1	+	19.4	0.0
B. ciliatus	No plants	....	....	6.9	22.3	+	8.2	6.3	+	7.6	0.0
Hordeum jubatum	6.2	66.7	+	9.7	0.0	−	13.1	0.0	−	15.1	0.0
Avena fatua	33.5	0.0	−	28.7	18.7	+	39.6	0.0	−	36.5	0.0
Phleum pratense	15.2	0.0	−	14.1	0.0	−	19.2	0.0	−	20.3	0.0

TABLE V

REACTION OF CERTAIN GRASSES TO *O. graminis*, *H. sativum* AND *F. graminearum* IN UNSTERILIZED SOIL

Organism	*Ophiobolus graminis*			*Helminthosporium sativum*			*Fusarium graminearum*			Check—no organism	
Host species	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %	Results of isolation trials	Av. plant height, cm.	Av. degree of infection, %
A. cristatum	5.3	80.0	+	No plants	....	....	13.2	0.0	−	12.7	0.0
A. sibiricum	7.2	20.0	+	12.8	10.0	+	16.6	0.0	−	14.6	0.0
A. obtusiusculum	4.3	100.0	+	20.1	20.0	+	17.2	0.0	−	22.5	0.0
A. smithii	No plants	....	....	10.0	20.0	+	No plants	....	....	12.5	0.0
A. desertorum	2.0	100.0	+	No plants	....	....	12.5	0.0	−	16.1	0.0
A. caninum	5.0	89.6	+	12.0	20.0	+	19.0	0.0	−	18.0	0.0
A. elongatum	Not seeded	....	....	No plants	....	....	20.0	0.0	−	20.7	0.0
A. richardsonii	8.9	89.6	+	12.9	22.4	+	15.7	0.0	−	13.6	0.0
A. griffithsii	5.3	60.0	+	13.6	10.9	+	12.4	0.0	−	11.4	0.0
A. tenerum	5.6	96.0	+	17.2	26.7	+	15.3	0.0	−	14.4	0.0
A. dasystachyum	4.8	93.3	+	8.8	40.0	+	14.4	0.0	−	14.0	0.0
A. repens	8.3	64.6	+	13.9	20.0	+	19.1	0.0	−	16.4	0.0
Bromus inermis	5.3	92.0	+	14.4	33.6	+	13.2	20.0	+	15.7	0.0
B. ciliatus	3.2	100.0	+	6.5	33.3	+	7.7	11.4	+	7.6	0.0
Hordeum jubatum	No plants	....	....	10.7	6.4	−	11.5	0.0	−	10.5	0.0
Avena fatua	27.6	0.0	−	26.1	6.0	−	27.6	6.0	+	23.1	0.0
Phleum pratense	10.5	0.0	−	11.7	0.0	−	15.7	0.0	−	11.3	0.0

In many cases a high percentage of the seedlings was killed before emergence by *F. graminearum*, and there were very few cases in which this organism was isolated later in the life of the plant. Plating resulted in re-isolation of the organism from plants grown either on sterilized soil or on unsterilized soil, in the species *Agropyron elongatum*, *A. repens*, *Bromus inermis*, *B. ciliatus* and *Avena fatua*. Only the two species of *Bromus* yielded the organism when grown on both sterilized and unsterilized soils. *Hordeum jubatum* showed no infection with *F. graminearum*. *Phleum pratense* was the only species resistant to all the organisms studied under the conditions of the experiment.

Relation of Western Rye Grass to the Amount of Take-all in the Following Wheat Crop

In 1927 the Division of Plant Biochemistry of the Department of Field Crops* at the University of Alberta began an experiment to determine the effect of alfalfa, western rye grass, brome and timothy on wheat yield and quality. Three series were laid out, each series having four replicates of each crop arranged as a Latin Square, to be broken in 1928, 1930 and 1932. The second series was plowed in 1930 and seeded to wheat in 1931. It was noticed that a large amount of take-all was present in the wheat on some plots, and counts of take-all were therefore made in order to obtain an estimate of damage. The average damage due to take-all following western rye grass was 23%, and on the plots of wheat after brome grass only 7%. There was no appreciable damage to the wheat following timothy and alfalfa.

In the third series the western rye grass appeared to be dying out in 1931, and in 1932 so little western rye grass was left that the plots were no longer suitable for their original purpose. By this time they had become over-run with weeds, such as dandelions and numerous grasses (Fig. 2).

As it was considered that the killing of the western rye grass might be due to take-all, roots of this grass from each of the plots were plated out at the end of May, 1932. *O. graminis* was isolated from roots from all four replicate

TABLE VI

INFECTION OF WHEAT WITH TAKE-ALL AND FOOT ROT WHEN GROWN ON SOILS AFTER DIFFERENT CROPS

Type of plot from which soil was obtained	Number of pots of wheat grown	Average plant height, cm.	Av. % degree infection of wheat with *O. graminis*	Av. % degree infection with other organisms
Western rye grass	20	26.1	4.8	6.2
Brome	8	26.4	0.0	3.3
Timothy	8	25.1	0.0	4.0
Alfalfa	8	26.9	0.0	4.5
Summerfallow	10	28.5	0.0	5.5
Sterilized soil	10	27.8	0.0	0.9

*Under the direction of Dr. R. Newton.

plots of western rye grass. *H. sativum* was also isolated, but symptoms of injury of plants by *O. graminis* were far more marked than were any other symptoms. No sign of take-all appeared on the brome or timothy plants. In order to determine the abundance of *O. graminis* in the soil from these plots and on soil growing other grasses or being summerfallowed, twenty five-inch pots were filled with soil from the western rye grass plots together with any stubble that may have been in the soil, eight each from the brome, timothy and alfalfa plots, ten from an adjacent summerfallowed soil, and ten with sterilized black soil. All were seeded to wheat, which was harvested after 21 days. Each plant was measured and the infection with *O. graminis* and with other organisms estimated. The data are given in Table VI.

The results summarized in Table VI show that *O. graminis* was isolated only from soil which had previously grown western rye grass. It appeared that in all probability other wheat foot-rotting organisms were also more abundant after western rye grass, but attempts to isolate them were not made. These results are further evidence of the part played by this grass in the problem of foot rot in wheat. The survey made seemed to show that there is a fairly general tendency for the older fields of *A. tenerum* to be more severely damaged by foot-rotting organisms, and it would be of value to determine if this is generally the case.

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A STUDY OF VARIOUS MEASURES OF VISCOSITY OF FLOUR-WATER SUSPENSIONS IN RELATION TO QUALITY[1]

By R. K. Larmour[2] and H. R. Sallans[3]

Abstract

Viscosity measurements were made with the MacMichael viscosimeter on leached and unleached suspensions of 11 experimentally milled flours representing the range 8.2–18.3% protein. The leached flour values gave the least satisfactory differentiation. The actual measurements made on unleached suspensions, or the response to acidulation or to increased flour concentration of the unleached suspensions, gave as much differentiation as the bromate baking test. Of the latter three the simple determination of viscosity of the unleached suspensions is recommended as the most rapid.

Most of the tests of flour quality, with the exception of ash and nitrogen, require a great deal of skill and judgment on the part of the operator. Because these attributes cannot readily be standardized, many modifications of the principal tests have been introduced from time to time and the confusion arising from the variations in the technique of individual workers and the resulting differences in data and in interpretation of data has been the despair of those interested in placing wheat and flour testing on a quantitative basis. The work of Sharp and Gortner (7) in 1923 stimulated interest in the viscosity of flour-water suspensions as a means of estimating flour quality because it seemed possible to subject this method to accurate control and thus establish a test that would be easily replicated by different workers. This, however, was not realized and at present the viscosity test is used very little in routine work. Its disuse is attributable to a number of causes, the principal of which are: first, the method recommended by Sharp and Gortner (7) and later by Johnson and his coworkers (2, 3, 4) involves the leaching of the salts from the flour, and this requires nearly as much time as the baking test, and secondly, the removal of electrolytes makes the colloidal system so sensitive to the influence of conditions that it is difficult to obtain reproducible results. Any method designed to replace the baking test, wholly or in part, must be more rapid or more precise or both; the determination of viscosity of leached flour-water suspensions meets none of these requirements and it is not surprising therefore that it has found little acceptance in routine work.

It seems likely that the baking test, despite its lack of standardization and the great number of slightly understood factors that affect it, will remain, for some time to come, the criterion by which other tests of quality must be judged. Modifications of the baking test have been proposed from time to

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time by various investigators but it is extremely difficult to produce evidence that one procedure or formula is better than another. About the only possible basis on which baking procedures can be compared is "commercial valuation" and as this factor cannot be accurately measured there are always certain to be differences of opinion as to how well a given formula interprets the millers' and bakers' estimates of flour values.

In dealing with western Canadian wheat, the main problem is to estimate the potential strength of flours rather than their value for bread-making *per se*, because most of this wheat is exported to be used in blends with softer wheats and its real worth depends mostly on ability to make up quality deficiencies. During the past few years a number of extensive studies of various baking formulas have been made with particular attention directed to the effect of potassium bromate. Geddes and Larmour (1) discussing the available evidence concluded that the bromate formula is much superior to the basic formula of the A.A.C.C., especially when applied to the evaluation of Canadian hard red spring wheat. In view of the fact that most of the earlier work on viscosity had been referred to baking results obtained by the basic formula it was thought advisable to check the viscosity method against the bromate baking method to see how closely the results were correlated. It was particularly desirable to learn how closely viscosity of unleached flour suspensions would prove to be related to the baking results by the bromate formula.

Material

The material chosen for this study consisted of 11 composite samples of flour experimentally milled from pure strain Marquis of the 1929 crop. These flours had a protein range of 8.2 to 18.3% and were quite representative of flours from the Saskatchewan hard red spring wheat crop of that season. The number of samples in each composite flour, the protein and ash contents, and the more important baking data are given in Table I.

A few words need to be said here regarding the composition of this series. In connection with another study samples of pure strain Marquis were collected from representative places in the province. After milling and baking tests had been made on the individuals, the residues of flour were combined into these 11 samples, the only selection exercised being that of putting flours of about the same protein content (using 1% ranges) into the same lot. At the extremes of the series the number of samples was, of course, rather small, but for protein values of 10–16% inclusive there were not fewer than 11 individual flours in each composite. Using this same series Larmour and Brockington (6) showed that the baking data of the composites corresponded very closely with the averages of the results obtained with the individuals. It was concluded therefore that little, if any, complementary effect occurred as a result of the mixing.

Attention should be directed to the fact that whereas there is a very wide range of protein values, the simple or basic formula gave relatively small differentiation of the samples, while the bromate formula gave results in

nearly the same order as the protein content. As far as we can appraise it, "commercial values" for blending purposes are in about the same order as protein. This is partially confirmed by the fact that baking results with a soft-flour-blend formula fall in this order also.

Experimental

The viscosity measurements were made with the new type MacMichael viscosimeter using a No. 30 wire, a 2-cm. bob, and the 5-cm. bowl revolving at 100 r.p.m. All data are recorded in degrees MacMichael and for sake of brevity these values will be referred to as the viscosity.

TABLE I

ANALYSES AND BAKING DATA OBTAINED WITH THE COMPOSITE MARQUIS SERIES

Flour no.	No. of samples in composite	Protein of flour, %, 13.5% mb.	Ash in flour, %, 13.5% mb.	Simple formula		Bromate formula	
				Loaf vol., cc.	Score	Loaf vol., cc.	Score
1	6	8.2	0.53	505	60	495	66
2	5	9.2	0.53	515	68	525	77
3	11	10.0	0.52	543	75	568	88
4	15	11.1	0.44	573	87	618	100
5	12	12.1	0.51	580	87	670	112
6	28	13.3	0.45	583	86	725	124
7	22	14.0	0.46	625	94	735	126
8	14	15.0	0.54	628	92	815	140
9	12	16.0	0.52	580	84	770	131
10	8	17.1	0.51	560	77	800	137
11	5	18.3	0.58	618	90	865	149

Two series of determinations were made, one on unleached and the other on leached flour-water suspensions, using four concentrations of flour in each. In the unleached series four concentrations of lactic acid were used on each sample, namely 0.5, 1.5, 2.5, and 5.0 cc. of 20% acid per 100 cc. of flour suspension. In the leached series, concentrations of 0.1, 0.3, and 0.5 cc. of 20% acid per 100 cc. of suspension were used. The unleached flour suspensions were prepared by adding 100 cc. of carbon dioxide-free distilled water to the weighed sample in a 250-cc. Erlenmeyer flask and shaking at five-minute intervals for one hour. The leached flour-suspensions were prepared by adding one litre of carbon dioxide-free distilled water to the weighed sample in a one-litre Erlenmeyer, shaking vigorously at 5-min. intervals for 45 min., allowing them to settle for 15 min. and decanting the supernatant liquid; this was repeated twice, but in the second and third extractions the flasks were shaken for only 15 min. and the suspension allowed to settle for 15 min. All work was done in a constant temperature room at 24.5° C.

In making determinations with the various acid concentrations the acid was added in successive increments. For example, with flour No. 1, 26 gm.

per 100 cc., Table II, after the sample had been soaked for one hour, 0.5 cc. of 20% lactic acid was measured into the flask and after thorough shaking, the suspension was poured into the viscosimeter bowl and the reading taken; then an additional 1 cc. of lactic acid was run into the bowl and stirred by means of a glass rod while the bowl was rotating. After two minutes the reading "26° MacMichael" was obtained. This is recorded as the value for 1.5 cc. of lactic acid. After the addition of another 1 cc. of acid the reading "31° MacMichael" was obtained and recorded for 2.5 cc. of acid. This procedure appeared justified by the results of a rather extensive preliminary investigation which showed that with unleached suspensions, the values obtained by adding in a single dose or by increments were practically identical and that with leached samples, although the single dose usually gave higher values, the viscosity-acid curves were nearly parallel except with low concentration of acid in which case they tended to coincide.

TABLE II

VISCOSITY (EXPRESSED IN DEGREES MACMICHAEL) OBTAINED WITH UNLEACHED FLOURS OF THE COMPOSITE MARQUIS SERIES

Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added: 0.5	1.5	2.5	5.0 (Viscosity readings, degrees MacMichael)	Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added: 0.5	1.5	2.5	5.0 (Viscosity readings, degrees MacMichael)
1	17	8	9	10	13	7	17	37	48	52	59
	20	12	15	17	19		20	40	67	81	89
	23	16	21	24	27		23	48	93	105	116
	26	21	26	31	35		26	60	144	156	177
2	17	16	20	22	18	8	17	42	48	55	63
	20	20	26	28	25		20	42	70	82	101
	23	26	35	44	41		23	51	106	127	141
	26	31	55	66	62		26	50	141	164	186
3	17	17	22	24	27	9	17	28	47	59	72
	20	23	29	34	40		20	34	72	91	106
	23	28	42	46	52		23	43	108	132	152
	26	29	59	68	76		26	46	159	186	209
4	17	19	26	30	35	10	17	28	61	74	66
	20	24	40	48	56		20	32	86	112	124
	23	29	56	68	81		23	37	124	156	178
	26	35	70	94	108		26	44	172	209	231
5	17	17	32	35	42	11	17	38	63	82	90
	20	37	53	55	60		20	49	106	124	139
	23	38	71	75	88		23	59	143	164	186
	26	47	98	112	126		26	62	190	221	247
6	17	31	38	46	52						
	20	35	56	71	85						
	23	41	85	103	122						
	26	44	110	126	153						

NOTE:—*100 r.p.m.; No. 30 wire; 2-cm. bob; 5-cm. bowl; room temperature, 24.5° C.*

TABLE III

VISCOSITY (EXPRESSED IN DEGREES MACMICHAEL) OBTAINED WITH LEACHED FLOURS OF THE COMPOSITE MARQUIS SERIES

Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added			Flour no.	Weight of flour used, gm.	Cc. of 20% lactic acid added		
		0.1	0.3	0.5			0.1	0.3	0.5
		Viscosity of flow, degrees MacMichael					Viscosity of flow, degrees MacMichael		
1	11	32	35	33	7	11	110	125	117
	14	60	68	68		14	240	245	242
	17	125	130	122		17	265	330	320
	20	155	170	160		20	455	530	510
2	11	52	52	48	8	11	130	150	145
	14	98	102	95		14	210	252	241
	17	165	170	160		17	307	362	350
	20	230	252	232		20	337	342	331
3	11	68	69	62	9	11	115	151	149
	14	125	125	120		14	147	253	258
	17	197	222	235		17	302	397	385
	20	286	340	340		20	430	525	517
4	11	82	91	86	10	11	160	180	179
	14	147	158	160		14	222	274	272
	17	300	308	285		17	326	422	428
	20	357	390	370		20	389	557	549
5	11	95	99	90	11	11	150	184	185
	14	182	192	178		14	243	295	296
	17	307	340	325		17	325	417	419
	20	440	462	470		20	473	567	551
6	11	130	138	127					
	14	225	245	262					
	17	312	392	396					
	20	445	465	455					

NOTE:—*100 r.p.m.; No. 30 wire; 2-cm. bob; 5-cm. bowl; room temperature, 24.5° C.*

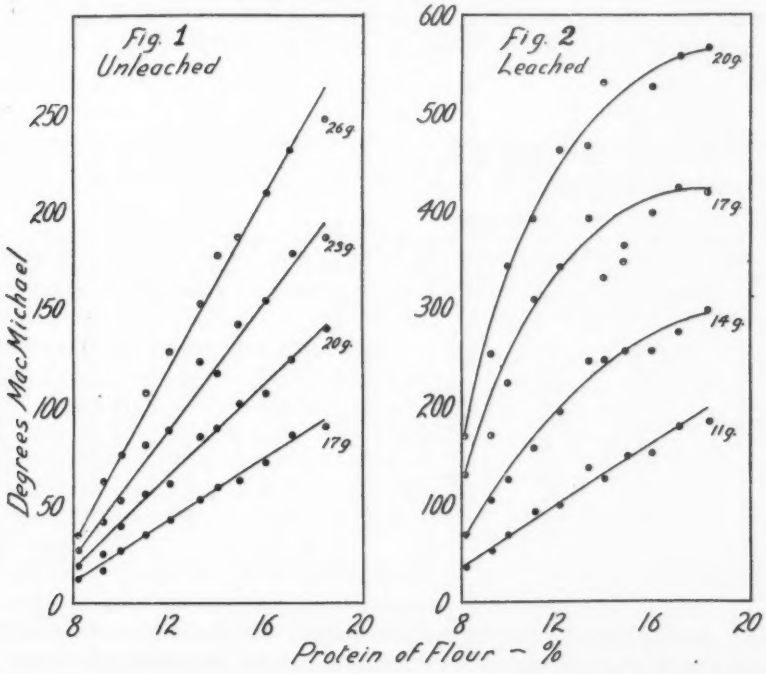
Discussion of Data

The data obtained with the unleached and leached suspensions are given in Tables II and III respectively. As four variables, namely, viscosity, protein, flour concentration and acid concentration are involved it is somewhat difficult to see their relation from the tables and it will be more convenient therefore to discuss the graphs.

Relation of Viscosity and Protein Content

In Figs. 1 and 2 the viscosities are plotted against protein content of flour using the values obtained with 5 cc. and 0.5 cc. of lactic acid solution on the unleached and leached samples respectively. With the unleached samples the relation of viscosity to protein was linear for all concentrations used, whereas with the leached flours only the lowest concentration, 11 gm., gave a linear relation, the other three being decidedly curvilinear. This curvili-

nearity, which is more pronounced at higher than at lower flour concentrations, means that the low-protein flours were more highly differentiated by the viscosity values than the high protein flours. Furthermore, all the curves in Fig. 2 tend to be linear for the lower values. It appears reasonable therefore to assume that the curvilinearity of these graphs is due to errors involved in trying to measure the highly concentrated, high-protein suspensions. At values of about 250° MacMichael and greater, the suspensions get very thick and in all probability are quite plastic. Suspensions for which values of 500° MacMichael are recorded show little tendency to flow and it seems very likely that they have a sufficiently high plasticity to render the readings obtained by the torsion instrument quite invalid.



FIGS. 1 AND 2. *Relation of protein of flour and viscosity of unleached and leached suspensions.*

The interesting point brought out by these graphs is that when dealing with flour concentrations so low that the maximum viscosity does not exceed about 250° MacMichael, one observes a linear relation between protein content and viscosity with both leached and unleached flours. It should be noted too that the curve for the 11-gm. leached flour concentration practically coincides with that for the 23-gm. unleached flour concentration, using, of course, different acid dosages in the two cases. In other words, by selecting a certain flour and acid concentration, it was possible to get exactly the same

results with unleached as with leached flours. It should be added here that determinations made on leached flours of 10- and 12-gm. concentrations respectively also gave results that showed a perfectly linear relation to flour protein.

Relation of Viscosity to Flour Concentration

In studying the relation of flour concentration to viscosity it has been customary to consider the logarithms of the two variables rather than the actual values, because the latter usually give curves of the exponential type. Sharp and Gortner (7) found that the tangent of the angle made by the logarithmic curve with the axis of abscissa was related to the strength of the flour. This constant, designated b, was calculated by the method of least squares and also by estimation from the plotted values. Data for the different samples are given in Table IV and the curves are shown in Fig. 3.

TABLE IV

COMPARISON OF GORTNER'S CONSTANT b AS OBTAINED FROM THE GRAPHS AND BY CALCULATION, USING LEACHED AND UNLEACHED FLOURS

Flour no.	Unleached flours				Leached flours			
	2.5 cc. 20% lactic		5.0 cc. 20% lactic		0.3 cc. 20% lactic		0.5 cc. 20% lactic	
	Observed	Calcd.	Observed	Calcd.	Observed	Calcd.	Observed	Calcd.
1	2.900	2.766	2.333	2.325	2.762	2.684	2.739	2.645
2	2.900	2.610	2.737	2.922	2.652	2.599	2.500	2.599
3	2.650	2.390	2.444	2.338	2.650	2.658	2.800	2.888
4	2.500	2.649	2.600	2.636	2.583	2.487	2.474	2.513
5	2.500	2.649	2.588	2.571	2.692	2.566	2.750	2.803
6	2.350	2.390	2.500	2.571	2.042	2.026	2.615	2.132
7	2.450	2.494	2.600	2.468	2.400	2.309	2.448	2.349
8	2.501	2.584	2.588	2.519	2.033	1.480	2.233	1.487
9	2.650	2.675	2.545	2.519	2.120	2.086	2.080	2.072
10	2.450	2.442	2.300	2.364	1.862	1.908	1.913	1.908
11	2.300	2.545	2.350	2.312	1.875	1.875	1.800	1.816

Considering first the unleached series, it appears that the value b is practically constant throughout the series of flours. This can be readily observed from Fig. 3 in which the curves are nearly parallel, necessitating a constant value for b. None of the individual values were significantly differentiated from the mean for the series; consequently we can consider that the slopes of the curves were constant. According to the postulate of Sharp and Gortner the value of b is a measure of a qualitative factor in the strength of flour proteins. Assuming their deduction regarding the significance of constant b one would be forced to conclude that no qualitative differences exist in the present series and that the strength of the flours is a direct function of the quantity of protein present.

Using protein of flour as a measure of strength, which according to the data given in Table I appears justifiable, it is evident that there is little relation

to the values of constant b. The correlation coefficient for these two factors was found to be $r = -.309$, which is not significant. As a means for differentiating the unleached flours constant b was of no value.

With leached flour-water suspensions somewhat different conclusions were reached. It can be seen that there was a tendency towards decrease in slope of the logarithmic curves as the protein of the flour increased. This is definitely shown by the values of constant b given in the lower half of Table IV. The values of b for 0.5 cc. of lactic acid correlated with protein of flour give the value $r = -.88$ indicating an inverse relation between these two variables. This would indicate that the relative quality of the protein decreases as the quantity increases. Obviously this is incompatible with the conclusion reached with unleached flour-water suspensions.

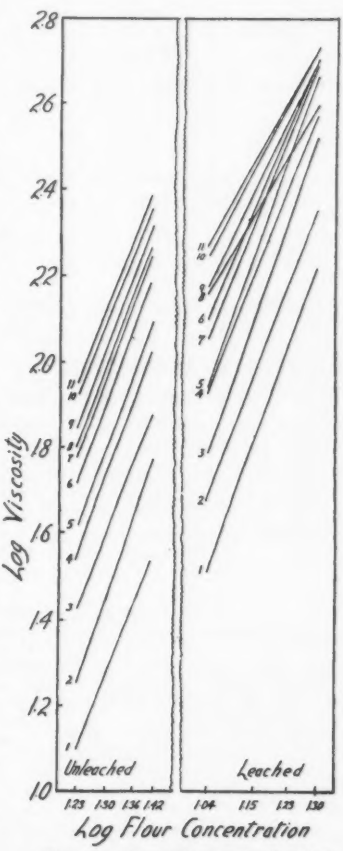


FIG. 3. *Comparison of unleached and leached suspensions.*

In the previous discussion of the data on which these values of b were based, it was pointed out that at a flour concentration of 13 gm. per 100 cc. or greater, the viscosity-protein relation is curvilinear and that this curvilinearity becomes accentuated with increasing flour concentration. Consequently the values obtained on this leached series for constant b are of extremely doubtful value as a criterion of flour strength. It is suggested that viscosity readings higher than 250° may be unreliable, due to the plastic nature of the suspension. If this is true it is conceivable that in the lower viscosity ranges for leached samples the results might be similar, if not identical, for unleached suspensions of some particular flour concentration. In Table V are shown the data for the 11-gm. leached suspensions using 0.5 cc. of acid, and for the 23-gm. unleached suspensions using 5.0 cc. of acid. It will be observed that the values are in such close agreement that they might easily represent duplicates. Evidently, by employing appropriate concentrations of acid and flour virtually the same results can be obtained by either method. This being the case there appears to be no justification for employing the laborious and time-consuming procedure of leaching the salts from the flours prior to determining the viscosity.

TABLE V

COMPARISON OF VISCOSITY OF UNLEACHED 23-GM. SUSPENSIONS AND LEACHED 11-GM. SUSPENSIONS AT THEIR RESPECTIVE OPTIMUM ACID CONCENTRATIONS

Flour no.	11 Gm. leached, 0.5 cc. 20% lactic	23 Gm. unleached, 5 cc. 20% lactic	Flour no.	11 Gm. leached, 0.5 cc. 20% lactic	23 Gm. unleached, 5 cc. 20% lactic
	Viscosity, degrees MacMichael			Viscosity, degrees MacMichael	
1	33	27	7	117	116
2	48	41	8	145	141
3	62	52	9	149	152
4	86	81	10	179	178
5	90	88	11	185	186
6	127	122			

Relation of Viscosity to Baking Value

Throughout this discussion reference has been made to protein of the flour as the index of baking quality. This was done because in the series studied the relation of loaf volume and general baking quality to protein content was very close. This can be seen from the data in Table VI. It is well known that two variables, each correlated to a common variable, will be correlated themselves. Therefore, because loaf volume (bromate method) and protein showed a correlation of +.976 it follows that any series of values closely related to protein must also be closely related to loaf volume.

TABLE VI

FLOUR PROTEIN, LOAF VOLUME, AND VARIOUS VISCOSITY MEASUREMENTS

Flour no.	Protein of flour	Loaf volume, cc.		Constant *b* (observed)		Approx. slope of viscosity —flour conc. curve	Approx. slope of viscosity —acid conc. curve, 26 gm. flour	Observed viscosity values	
		Basic bromate		Unleached	Leached			26 gm. flour, 5 cc. acid, unleached	11 gm. flour, 0.5 cc. acid, leached
1	8.2	505	495	2.33	2.74	22	14	35	33
2	9.2	515	523	2.74	2.50	44	31	62	48
3	10.0	543	568	2.44	2.80	49	47	76	62
4	11.1	573	618	2.60	2.47	73	73	108	86
5	12.1	580	670	2.59	2.75	84	79	126	90
6	13.3	583	725	2.50	2.62	101	109	153	127
7	14.0	625	735	2.60	2.45	118	117	177	117
8	15.0	628	815	2.59	2.23	123	136	186	145
9	16.0	580	770	2.54	2.08	137	163	209	149
10	17.1	560	800	2.30	1.91	145	187	231	179
11	18.3	618	865	2.35	1.80	157	185	247	185

The viscosity data which may be considered as possible criteria of flour quality are: (1) the actual measurements as obtained by either the leached or unleached suspension; (2) the constant *b*; (3) the response to acidulation of the unleached suspensions; (4) the increases of viscosity due to increased

flour concentration. These data, together with protein and loaf volumes, are collected in Table VI. The correlations between these variables are shown by the coefficients given in Table VII.

It should be borne in mind that this series of flours was not a selection of individual samples chosen from the regression of loaf volume (bromate) on protein, but a series of composites made up on the basis of protein without regard to the baking results previously obtained on the individual samples.

TABLE VII

COEFFICIENTS OF CORRELATION FOR THE VARIABLES GIVEN IN TABLE VI

Variables	r
Protein x loaf volume (basic)	+ .733
Protein x loaf volume (bromate)	+ .976
Protein x constant b (unleached flours)	− .309
Protein x constant b (leached flours)	− .876
Protein x approx. slope of viscosity-flour conc. curve (unleached, 5 cc. acid)	+ .992
Protein x approx. slope of viscosity-acid conc. curve (unleached 26 gm.)	+ .994
Protein x actual readings on unleached suspensions, 26 gm. flour, 5 cc. acid	+ .997
Protein x actual readings on leached suspensions, 11 gm. flour, 0.5 cc. acid	+ .991
Loaf volume (bromate) x constant b (unleached flours)	− .221
Loaf volume (bromate) x constant b (leached flours)	− .791
Loaf volume (bromate) x approx. slope of viscosity-flour conc. curve (unleached)	+ .981
Loaf volume (bromate) x approx. slope of viscosity-acid conc. curve (unleached)	+ .963
Loaf volume (bromate) x actual readings on unleached suspensions; 26 gm. flour, 5 cc. acid	+ .978
Loaf volume (bromate) x actual readings on leached suspensions; 11 gm. flour, 0.5 cc. acid	+ .977

The close relation between protein and bromate loaf volume therefore cannot be considered fortuitous. As previously pointed out by Larmour (5) the relation between basic loaf volume and protein is curvilinear in the higher protein ranges, while with the bromate loaf volume it is essentially linear throughout the range. On this basis it seems justifiable to use the bromate loaf volume as the criterion by which to judge the usefulness of the viscosity data.

The increased viscosity due to increased flour concentration, and to increased acid concentration, the actual viscosity measurements made on 26 gm. unleached flour suspensions with 5 cc. of acid, and on 11 gm. leached flour suspensions with 0.3 cc. acid, and the protein of the flour were all highly and about equally correlated with bromate loaf volume and with each other. The constant b obtained with the unleached suspensions was not significantly correlated with any of the other variables. Constant b obtained with leached suspensions was highly correlated negatively with loaf volume and protein. If Sharp and Gortner's interpretation of the meaning of constant b is correct it follows that the quality of these flours is constant, if the data from the unleached flours is taken, and this figure therefore cannot be used to differentiate the flours in such a series.

Of the other measurements, the simple determination of viscosity of an unleached suspension is obviously the most rapid and as it gives as much differentiation as any of the others, is to be preferred.

In conclusion it should be pointed out that this study was conducted with sound samples of one variety grown in a single season and although the conclusions reached are well supported by the data, there is no indication of what may be expected with different varieties, different seasons or various forms of damage. This is to be the subject of further investigation.

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES[1]

By P. H. H. Gray[2] and N. B. McMaster[3]

Abstract

Microbiological studies of samples from the separate horizons and from different depths of soils of the Appalachian upland podsol group show that the activity of the micro-organisms is dependent upon the organic-matter relations in the horizons. The organic-matter horizon is biologically the most active, as shown by analyses for carbon dioxide, nitrate nitrogen, numbers of bacteria, and production of ammonia from urea. Evidence is submitted that the reduced activity of the leached layer and the horizons of accumulation is not due to toxic compounds produced by leaching of the organic matter.

Characteristic profiles of virgin podsol soils in the province of Quebec have been recently described (13, 14). The chief factor that brings about the partition of the soil profile into easily recognizable horizons appears to be the accumulation of a surface mulch of raw or semi-decomposed organic matter that holds moisture and, owing to its acid nature, is the cause of the chemical displacement of basic fertility elements to lower depths of the soil by leaching. In the presence of this high content of potential energy and body-building material the micro-organic population of the virgin soils seems to be at a low level of activity in comparison with that of soils of other groups. Agricultural soils of the upland podsol group that have been cultivated for many years are characterized by their light brown color, in spite of their great content of organic matter, and by their lack of fertility except with continual applications of organic matter and mineral fertilizer. Analyses of three typical cultivated soils show an average total organic carbon of 3.96%, and an average total nitrogen of 0.27% in the surface six inches. Infertility, indeed, as pointed out by Bizzell (4), may be associated with a high content of organic matter in cultivated soils. It has been a matter for discussion whether the lower level of activity in these soils is due to the higher hydrogen ion concentration, the removal of basic nutrients, or to some other cause, such as the accumulation of compounds toxic to the micro-organisms more directly concerned in soil fertility.

The removal of basic nutrients to lower levels of soil suggested that the bacterial flora concerned in reactions important in plant nutrition would be adversely affected either by lack of food material or by the production of acidic or other substances toxic to these organisms. It seemed, therefore, to be of first importance to analyze the distinctive horizons in virgin soils of this nature, to ascertain their biological activity and some at least of the factors affecting that activity. Such studies would appear to be of greater value if a limit having some reference to field practice were set to the depth

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of soil from which samples should be taken. In the studies previously made certain biological aspects of the surface eight inches of virgin soils were reported. In a sample of that depth the proportions of the different horizons vary in different soils; variation in thickness of horizons also occurs in subsamples of the same soil (see Fig. 1). In the present work a depth of 12 in. was chosen, in order to include part at least of the horizon that receives a large proportion of the leached constituents. This limit of depth for sampling had a certain value in that the soils selected for this study adjoined cultivated soils of the same type in which experiments were in progress to ascertain the effects of ploughing to a greater depth than was customary. Deep ploughing has been recommended (14, 15) as a remedial practice for recovering the lost fertility of soils of this nature. Whether the biological action on the organic matter is at a higher level in the cultivated soils has not yet been ascertained.

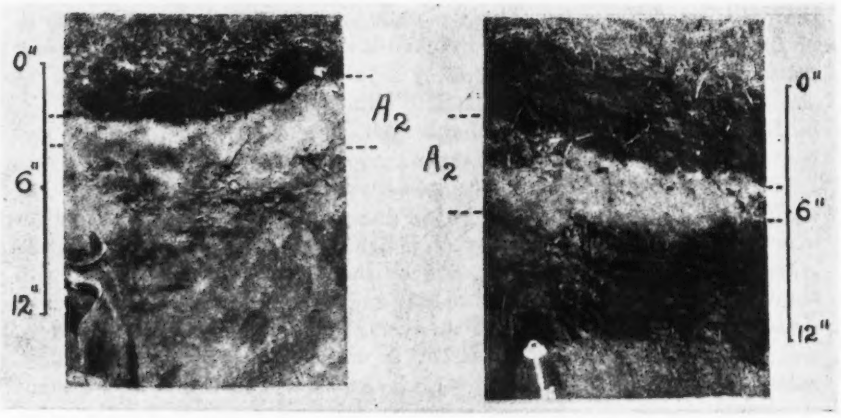

FIG. 1. *Representative profiles of upland podsol soils. The thicknesses of the A_2 (leached) horizons and the depths of the profiles are indicated.*

The present paper is an introduction to the study of the biological conditions in the horizons of representative virgin soils of the Appalachian upland podsol group. In addition, studies have been made upon the biological activity of freshly mixed horizons to depths of 6 and 12 in., to determine the relative activity in soil to these depths and to provide a basis for comparison with the same soils under cultivation.

Soils and Soil Sampling

The virgin soils selected for these studies were three from the Eastern Townships region of Quebec, that were adjacent to cultivated soils in the same area upon which investigations of a similar nature were in progress. A description of the soil profiles is given in Table I.

TABLE I

DESCRIPTION OF SOIL PROFILES

Sample	Ecology	Depths of horizons, and description
S	Permanent pasture, 1250 ft. above mean sea level; near maple bush; moss hummocks prevalent	A_1, 1–2 in., dark brown humus and semi-decomposed residues A_2, ½–3 in., ashy-grey to dark grey leached fine sandy loam B, 3–6 in., reddish brown sandy loam, shading to yellowish brown sandy loam, with some stones, down to 12 in.
R	Permanent pasture, 675 ft. above mean sea level; *Thuja* and other evergreens; moss hummocks	A_1, 1–2½ in., dark brown to black mucky fine sandy loam A_2, 1–1½ in., ashy leached fine sandy loam B, 5–6 in., reddish brown fine sandy loam, shading to greyish brown fine sandy loam down to 12 in. About 1 in. of the C horizon is included in B.
B	Woodlot, 675 ft. above mean sea level; mixed coppice, birch predominant	A_1, 3–5 in., dark chocolate-colored fine sandy loam A_2, 1–1½ in., ashy leached fine sandy loam B, 1–3 in., reddish brown fine sandy loam, shading to light reddish yellow fine sandy loam at 12 in.

In the above description no attempt is made to differentiate between sub-horizons of the B (alluvial) horizon.

Samples were taken at different seasons during 1931, as shown in the succeeding tables of results. Samples were obtained from the separate horizons by means of a flat spade, the final separation being done by hand. Horizon B was sampled down to a depth of 12 in. from the surface. To obtain samples of the two depths, the soil was sliced vertically to the required depth, and different slices thoroughly mixed by hand.

Analytical Methods

Moisture. Moisture in the fresh samples and hygroscopic moisture in air-dried samples were determined by the standard methods of the Association of Official Agricultural Chemists (1). The water-holding capacity of the air-dried samples was determined by the method previously described (13); tests were made to compare this method with that described as the "box" method (11), and close agreement was obtained; the latter method was found, however, to be too lengthy a procedure for the number of samples involved. The degree of saturation of the fresh soil, on a basis of moisture-free soil, was calculated from the equation

$$s\,(\%) = \frac{m - h}{c} \times 100,$$

in which m is the moisture in fresh soil, h is the hygroscopic moisture and c is the water-holding capacity of air-dried soil. The moisture relations, together with the loss on ignition, are shown in Table II. All results, in this and later tables, are reduced to a basis of moisture-free soil.

Hydrogen ion concentration. The pH of fresh samples from the three horizons was determined by the bubbling hydrogen electrode, using 3.5 gm. of air-dried soil in 12.5 cc. of distilled water.

TABLE II

MOISTURE AND LOSS ON IGNITION IN SAMPLES FROM SOIL HORIZONS AND DIFFERENT DEPTHS (EXPRESSED AS PERCENTAGES ON A BASIS OF MOISTURE-FREE SOIL)

Soil	Date sampled 1931	Horizon	Moisture in fresh sample, %	Hygroscopic moisture, %	Loss on ignition, %	Water-holding capacity, %	Moisture in fresh sample, as per cent of water-holding capacity
S	Oct. 23	A_1	97.38	8.09	42.38	101.50	87.89
	Oct. 23	A_2	18.74	0.82	2.07	37.52	65.12
	Oct. 23	B	34.97	3.84	7.93	47.77	65.18
R	Sept. 29	A_1	207.50	12.14	60.16	152.50	128.10
	Sept. 29	A_2	34.41	2.44	5.85	51.74	61.80
	Sept. 29	B	52.41	3.02	5.96	58.20	84.86
		Depth, in.					
S	Aug. 4	0– 6	44.73	3.67	10.19	63.75	64.40
	Aug. 4	7–12	31.69	3.15	7.19	50.81	56.16
	Sept. 15	0– 6	23.25	3.26	5.48	50.59	39.52
	Sept. 15	0–12	24.69	2.41	4.90	49.16	22.82
R	Aug. 18	0– 6	43.53	3.67	14.05	57.80	68.98
	Aug. 18	0–12	29.51	2.56	6.61	48.20	61.51
B	July 21	0– 6	84.78	4.65	18.31	72.04	111.30
	July 21	0–12	70.89	4.05	14.72	66.06	101.30

Loss on ignition and *organic carbon.* These were determined by dry combustion methods (14).

Total nitrogen. Total nitrogen was determined by the Gunning-Hibbard method as described by the Association of Official Agricultural Chemists (1).

The results of these determinations are shown in Tables II and III, as percentages on a basis of moisture-free soil; each value in these tables is a mean of duplicate determinations. The analyses which compose Table III were carried out in the Chemistry Department, Macdonald College.

Carbon dioxide. The evolution of carbon dioxide from fresh samples was determined as previously described (13). Usually, 500 gm. of soil was

TABLE III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	pH	Carbon (organic), %	Total nitrogen, %	Ratio C : N
S	A_1	5.23	17.060	0.666	25.6
	A_2	4.63	0.566	0.039	14.5
	B	5.15	3.468	0.190	18.3
R	A_1	5.10	31.340	1.308	23.9
	A_2	5.25	0.617	0.033	19.0
	B	5.46	2.208	0.143	15.4

placed in the container, and the gas collected at intervals for periods varying from 286 to 400 hr. Tests were also made for variation among triplicate subsamples, and agreement was found to be close in the case of eight samples. The results are expressed as mgm. of carbon dioxide per 100 gm. of moisture-free soil per 100 hr.

Nitrate nitrogen. Nitrate nitrogen was determined by Harper's modification of the phenol-disulphonic acid method (10). The soils were incubated with the original moisture at room temperature, in glass jars that permitted aeration but allowed a minimum of evaporation. The results, which represent nitrification of the soil's own nitrogen, have been expressed as parts per million of moisture-free soil. A test for variation was made with triplicate subsamples of one sample, and close agreement was found.

Bacterial numbers. Bacteria and actinomyces in fresh samples were counted by the plate method, using Thornton's medium (16). A set of five plates was prepared from each sample; in some cases, however, the numbers could be calculated only from a smaller number of plates; the number of plates used, and the reliability of the results in relation to the χ^2 index of dispersion, are shown in the tables of results (Tables VI and XI).

Soil Horizon Extract Media

Soil solution was extracted from the three horizons A_1, A_2 and B, of soil *S* by means of a displacement method. The samples from each horizon were dried and the water-holding capacity of each was determined. They were then remoistened to about 60% of the water-holding capacity, and packed with gentle but firm pressure into percolation cylinders, in which they were allowed to stand for a short period. Distilled water was then used to form a head of pressure, 3 to 4 in. deep, above the moist soil. The first few cubic centimetres of displaced liquid was discarded, and an amount of soil solution equal in volume to about 75% of the calculated true solution was collected. Clear light-amber colored liquids were thus obtained from each of the three horizons. With these extracts as the solvents, three agar media were prepared from each; to one portion nothing was added except the agar; to a second 0.1% of mannitol was added as a source of carbon; and to a third 0.05% of potassium nitrate was added as a source of nitrogen. A sample of soil *S* was plated on each of these media, and on Thornton's count medium. In the dilution fluid sufficient phosphorus, as dipotassium hydrogen phosphate, was added to give to the extract media in the plates an amount comparable with that in Thornton's medium. The results are shown in Table VII, in which the figures represent the mean number of bacterial colonies in four (in one case, three) plates.

Production of Ammonia from Urea

A mass of air-dried soil equivalent to 5.0 gm. of moisture-free material was inoculated into 50 cc. of a solution consisting of urea, 5.00%, and dipotassium hydrogen phosphate, 0.05% in distilled water in Hansen yeast culture flasks, and the cultures incubated at 25° C. in a room the temperature

of which was controlled by a thermostat. The amount of ammonia was titrated at intervals with $N/14$ sulphuric acid, using methyl red as the indicator. The results, expressed as mgm. of ammonia nitrogen per cc. of culture, are given in Tables VIII and XII. Ammonia found in an uninoculated flask of the same medium amounted to 0.61 mgm. per cc. This has been subtracted to give the values quoted.

Discussion of Results

A. The Separate Horizons

The biological activity of the separate horizons is shown by the amount of carbon dioxide evolved to be greatest in the organic-matter horizon of both soils of which the separate horizons were studied (see Table VI). The ratio of the amount evolved by the A_1 horizon to that evolved by the leached layer is in the order of 25 to 1 in both soils. There is little or no difference between the leached horizon and that below it.

TABLE IV

CARBON DIOXIDE EVOLUTION; SAMPLES FROM SOIL HORIZONS

Horizon	Carbon dioxide, mgm. per 100 gm. per 100 hr.	
	Soil *S*	Soil *R*
A_1	138.80	358.30
A_2	5.30	14.20
B	6.10	5.60

The results of the analyses for nitrate nitrogen in the separate horizons are shown in Table V. No nitrate

TABLE V

NITRIFICATION IN SAMPLES FROM SOIL HORIZONS

Soil	Days	Nitrate nitrogen, parts per million			Soil	Days	Nitrate nitrogen, parts per million		
		A_1	A_2	B			A_1	A_2	B
S	5	Trace	Trace	Trace	*R*	7	Nil	Trace	Trace
	24	86	Trace	Trace		29	307.6	17.6	8.7
	209	137.5	99.5	14.0		51	452.2	29.8	12.1
						233	694.4	66.3	23.5

nitrogen was found in any of the fresh samples, and in some cases only traces were found after 24 days of incubation. The activity of the nitrifying bacteria follows the same trend in the three horizons as that of the whole population, in that the organic-matter horizon is considerably more active than those below it. The rate of nitrification in the organic matter horizon of soil *S* fell off rapidly after the 24th day, only 52 p.p.m. of nitrate nitrogen being formed in 185 days, as compared with 100 formed in the leached layer during the same period. Nitrification was rapid in the organic-matter horizon of soil *R*, 307.6 p.p.m. being formed in 22 days, a daily average of 14 p.p.m.; the rate then fell to 6.5 p.p.m. daily during the next 22 days, and to 1.3 p.p.m.

daily between the 51st and the 233rd day. These results are not in agreement with those of Lunt (12), who obtained little or no nitrate nitrogen in the humus horizons of forest podsol soils of New England.

The degree of nitrification in the lower horizons is not proportionate to the amounts of total nitrogen in them.

The numbers of bacteria in the different horizons (see Table VI) appear to follow the same trend as the activity of the whole population and of the nitrifying bacteria. The numbers of bacteria in the leached horizons can be expressed as a percentage of those in the organic-matter horizons. In soil *S* this percentage was 3.02 and in soil *R* it was 3.44. It is interesting to note that the proportional figures for the evolution of carbon dioxide are 3.8 and 3.9% respectively.

Fraenkel (8) found that the reduction of the bacterial flora with increasing depth in virgin soils was sharply defined. Waksman (18), who gives references to this aspect of soil bacteriology, found that, in a forest soil in the air-dried condition, numbers at arbitrary vertical intervals were progressively less and that there was an association between numbers and the carbon and nitrogen content of the samples. The differences in carbon and nitrogen were, however, considerably less than those reported here (Table III). A comparison of the numbers in the three horizons and their respective carbon and nitrogen contents suggests that there is a direct, though not a close, relation between bacteria and organic carbon.

The numbers of actinomyces follow the same trend as the bacteria in the three horizons and in the other samples (see Tables VI and XI). The proportion of these organisms, expressed as a percentage of the total plate count, appears to be rather lower than that usually found in cultivated soils when plated on the same medium.

In order to determine if the bacterial numbers in horizons A_2 and B, as found by plating the fresh soil, were less than those in the organic-matter

TABLE VI

NUMBERS OF MICRO-ORGANISMS IN SOIL HORIZONS

Soil	Date of plating	Horizon	No. of plates used in counting	Mean of bacterial colonies	Index of dispersion	Bacteria per gm.	Actinomyces per gm.	Actinomyces, % of total
S Oct. 23	Oct. 26	A_1	3	117.0	Within normal	23,210,000	5,025,000	17.8
		A_2	3	614.0	Within normal	702,000	58,390	7.7
		B	1	700.0	Within normal	464,610	25,890	5.3
R Sept. 29	Oct. 9	A_1	5	176.0	Within normal	10,116,000	1,494,000	12.9
		A_2	5	280.0	Within normal	348,000	17,270	4.7
		B	5	93.4	Excessive	135,670	10,730	7.3

horizon on account of the proportionate amounts of available nutrients or due to the presence of toxic compounds in the soil solutions, a sample of soil *S* was plated on media prepared with the solutions extracted as described, and on Thornton's medium. The results are shown in Table VII.

TABLE VII

COLONIES ON SOIL HORIZON EXTRACT MEDIA

Extract from horizon	Extract alone	Extract with mannitol	Extract with nitrate
A_1	98.50	105.70	111.50
A_2	78.00	65.25	81.00
B	69.75*	61.75*	68.00

Colonies on Thornton's medium, 86.75.
*χ^2 *excessive.*

The extract from the organic-matter horizon either alone or with added carbon or nitrogen gave higher numbers than the medium used as control, but the difference is found to be not significant when the *t* test for significance is applied (7). The higher numbers of colonies on the extract medium from horizon A_1 with additional carbon or nitrogen are also not significantly different from the number obtained with the extract alone. By the same test there is no significance between the numbers obtained by the extracts from horizons A_1 and A_2 when used alone, but when carbon or nitrogen is added the numbers in the leached horizon are significantly lower in both cases. The numbers shown by the plates from horizon B are not significantly lower than those shown by the plates from the leached horizon. The numbers given by the extracts from the leached horizon and horizon B are also not significantly different from the number given by the control medium. These results would appear to confirm the suggestion that lower numbers in the lower depths of these soils are not due to soluble toxic compounds.

The ammonification of urea is dependent to some extent upon the initial numbers of bacteria, as shown by Drewes (6) and Viehoever (17), and upon the subsequent rates of multiplication. Thus a sample having fewer bacteria may produce less ammonia in the first few days, but as growth proceeds the numbers of organisms may, provided there is sufficient available carbon, increase to a maximum; the amount of ammonia finally equals that produced in a shorter time by a sample having a greater initial number of bacteria. Comparisons of the amounts of ammonia produced can therefore be best made during the early stages of the reaction, and they may serve as indexes of the availability of the carbon in the soil sample. The results of this test are shown in Table VIII.

The organic-matter horizons are considerably the most active in both soils. The hydrolysis was complete by the 7th day in the case of soil *S*, and by the 15th day in the case of soil *R*. The maximum was not reached in the case

TABLE VIII

AMMONIA PRODUCED FROM UREA; SOIL HORIZONS

Soil	Horizon	Ammonia nitrogen, mgm. per cc.						
		Days	*3*	*7*	*9*	*16*	*22*	*41*
S	A_1		20.17	27.15	25.46	24.66	25.28	24.04
	A_2		1.47	9.99	12.93	22.65	24.41	23.16
	B		1.08	7.40	9.86	15.60	17.36	16.12
		Days	*3*	*7*	*9*	*15*	*22*	*41*
R	A_1		6.66	24.62	26.44	28.26	25.48	25.79
	A_2		1.09	6.83	10.47	14.70	13.58	12.59
	B		1.62	6.75	8.70	12.51	14.66	15.23

of either of the leached horizons, or of the B horizons; the difference in activity between these horizons is only slight. These results suggest that soluble compounds of carbon present in the organic matter are in sufficient quantity to allow the bacteria to complete the reaction. The amounts of available carbon are apparently much less in the leached horizons and in the horizons of accumulation; the ammonia is not, however, proportionate to the amount of total carbon.

Viehoever (17) suggested that a urea-decomposing organism *B. probatus*, utilized the compound autotrophically when grown in pure culture, obtaining its carbon from the air and from mineral compounds, including the ammonium carbonate formed from the hydrolyzed urea, hydrolysis being effected by the sterilization of the medium. While many workers are agreed (9) that available carbon compounds stimulate the production of ammonia from urea, Beijerinck (2) and Bierema (3) have shown that soil organisms are not able to use the compound as a source of carbon and nitrogen. The different rates at which the bacteria in the three horizons utilize the compound suggest that the amount of available food material as well as the initial numbers must be effective in determining those rates. In view of the results obtained by plating on the media prepared from the separate horizons, it would appear that the number of bacteria is the chief factor in these different rates of ammonification. The possibility that the differences are due to toxic compounds in the lower layers of soil appears to be ruled out by the results of plating on the extract media.

The solutions resulting from the action of the ammonia on the organic matter in the samples in the culture flasks varied from light brown to dark brown, depending to some extent upon the amount of ammonia present; the depth of color was not, however, directly parallel with the degree of ammonification.

B. Samples from Different Depths

It might be assumed, as indicated previously, that these soils are relatively much richer in the virgin state than they become after cultivation, and that

treatment widely different from that normally practised should be recommended. One of these recommendations is that they should be ploughed to a depth sufficient to return to the soil the basic nutrients removed by leaching, on the assumption that the organic matter would thereby be more readily decomposed. Against this it is widely believed that such treatment results in a low level of fertility.

The studies reported in this part were made to determine the biological activity of samples of soil taken to depths of 6 and 12 in. from the same trenches as those from which samples of the separate horizons were taken. The purpose of these studies was to compare the effects of mixing the organic-matter horizons with different amounts of "mineral soil" from the lower horizons, and thereby to obtain some knowledge of the biological activity of virgin soils in comparison with that of the same soils after many years of cultivation.

TABLE IX

CARBON DIOXIDE EVOLUTION; SAMPLES FROM DIFFERENT DEPTHS

Depth, in.	Soil *S*	Soil *R*	Soil *B*
	Carbon dioxide, mgm. per 100 gm. per 100 hr.		
0– 6 (Aug. 4)	40.96 (1)*	—	—
7–12 (Aug. 4)	7.20 (2)	—	—
0– 6	22.90 (3)	51.83 (5)	74.53 (7)
0–12	12.80 (4)	22.23 (6)	45.87 (8)

** The values for triplicate subsamples are as follows: (1) 42.3, 40.0, 40.0; (2) 8.0, 6.8, 6.8; (3) 24.7, 22.1, 21.9; (4) 14.7, 12.1, 11.6; (5) 52.4, 52.3, 50.8; (6) 24.2, 22.4, 20.1; (7) 78.2, 73.1, 72.3; (8) 46.2, 45.8, 45.6.*

The degree of activity represented by the evolution of carbon dioxide in the upper 6 in. of soil and in the lower 7–12 in. can be compared for soil *S* only, sampled on Aug. 4. The amount of carbon dioxide in the upper layer is, as shown in Table IX, nearly six times the amount produced by the lower 6 in. of soil. The amount obtained, approximately 41 mgm., from the upper layer can be calculated as that which would be expected on a basis of proportionate amounts of the separate horizons in a sample to the depth of 6 in., in which the horizons do not vary much in thickness from an average of the values shown in the description of the soils. The amount obtained from the 7–12 in. sample, also, is not greatly different from the value obtained in the case of the B horizon, 6.1 mgm., as shown in Table IV.

In comparing the values obtained for the carbon dioxide evolved by the 6 in. and the 12 in. depths of soil, it is clear that dilution of the upper 6 in. with material from the B horizon effects more than a twofold reduction in activity in the case of soils *S* and *R*, and a somewhat less reduction in the case of the soil *B*. The amount evolved by the upper layer of soil *R* can be considered as approximately what would be expected on a basis of proportionate masses of soil from the horizons.

TABLE X

NITRIFICATION IN SAMPLES FROM DIFFERENT DEPTHS

Soil	Days	Nitrate nitrogen, parts per million		Soil	Days	Nitrate nitrogen, parts per million	
		0–6 in.	7–12 in.			0–6 in.	0–12 in.
S (Aug. 4)	2	nil	nil	*R*	1	trace	nil
	37	37.6*	trace		23	18.7	4.0
	50	62.1	13.9		36	33.1	9.4
	107	117.9	22.9		93	117.3	33.9
	289	159.2	24.5		275	159.2	66.0
		0–6 in.	0–12 in.				
S (Sept. 15)	1	nil	nil	*B*	2	nil	nil
	15	trace	nil		20	trace	trace
	65	35.7	16.1		64	45.7	32.4
	247	68.5	29.3		121	126.2	93.2
					303	178.6	162.8

* *In triplicate subsamples: 39.64, 36.67, and 36.41.*

In neither soil was there evidence of reduced activity other than that due to dilution.

The amount of nitrate nitrogen was greater in the upper, 0–6 in., samples throughout the period of incubation (see Table X). The lower degree of nitrification by the 0–6 in. sample of soil *S*, of Sept. 15, in comparison with the 0–6 in. sample of Aug. 4, is probably due to the soil being too dry at the time of sampling. It will be seen in the last column of Table II that the percentage of saturation of the soil sampled on Sept. 15 was 39.5%, while that of the other sample was over 60%. It would seem that drought rather than excessive moisture adversely affects the nitrifying bacteria in soils of this nature.

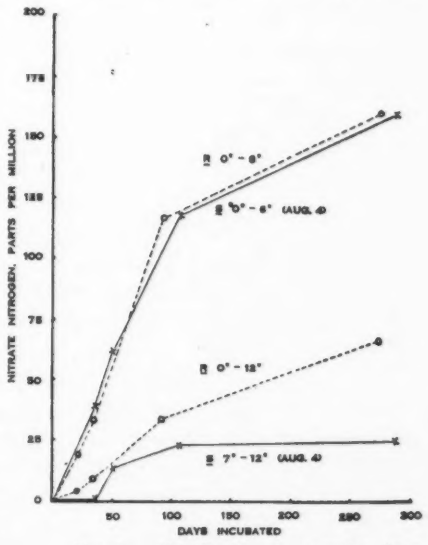

FIG. 2. *Nitrification in samples from different depths of virgin podsol soils.*

The results given for soils *S* (Aug. 4) and *R* are also shown in Fig. 2, wherein it will be found easier to compare the relative degrees of nitrification in the two pasture soils. There is close agreement between the 0–6 in. samples. Nitrification probably continued for some time after the final test in these two samples and in the 0–12 in. sample of soil *R*, but it ceased at about the 15th week in the lower (7–12 in.) sample of soil *S*. The other samples of soil *S* have been omitted from the graph for the reason stated.

The degree of nitrification in both layers of soil *B* would suggest that, in this soil at least, there are no compounds toxic to these organisms in the lower depth of the sample.

The numbers of bacteria and actinomyces are shown in Table XI.

TABLE XI

NUMBERS OF MICRO-ORGANISMS IN SAMPLES FROM DIFFERENT DEPTHS

Soil; Date	Date of plating	Depth of sample, in.	No. of plates used in counting	Mean of bacterial colonies	Index of dispersion	Bacteria per gram	Actinomyces per gram	Actinomyces, per cent of total
S Aug. 4	Aug. 5	0– 6	4	126.2	Excessive	818,800	163,400	16.6
		7–12	4	23.6	Within normal	145,530	28,370	16.3
S Sept. 15	Sept. 16	0– 6	3	522.0	Excessive	2,788,500	213,500	7.1
		0–12	4	266.0	Within normal	1,296,900	144,100	10.0
R Aug. 18	Aug. 19	0– 6	5	212.6	Within normal	1,546,500	374,500	19.5
		0–12	5	94.0	Within normal	674,300	232,900	25.7
B July 21	Aug. 12	0– 6	5	73.3	Within normal	643,640	86,160	11.8
		0–12	5	108.4	Excessive	970,000	114,800	10.6

The numbers found in the upper 0–6 in. samples are about twice those in the 12 in. samples in the case of those from soil *S*, of Sept. 15, and soil *R*. The variation shown by the two 0–6 in. samples of soil *S* may be due either to moisture in the soil, or to season. Cobb (5) has shown that numbers of bacteria and actinomyces in virgin forest soils of New York State fluctuate with season, and states that the moisture exercises the greater control. No evidence is available as to seasonal fluctuations in virgin soils under pasture.

The value shown for bacteria in the upper layer of soil *S* (Aug. 4) is not reliable since the value for χ^2 is excessive; the difference, however, between this value and that for the lower 6 in. is sufficiently great to show their approximate relation.

The numbers in the 0–12 in. sample of soil *B* are probably not significantly different from those in the 0–6 in. sample. The bacterial numbers and the nitrifying capacity of these two samples do not greatly differ, although the difference in the evolution of carbon dioxide is quite appreciable.

The production of ammonia from urea is shown in Table XII. The amounts on the third day appear to be comparable with the number of bacteria in the respective samples. The amounts approach equality on the 9th day, except in the case of the 0–6 in. and 7–12 in. samples of soil *S*, in which the amount produced by the upper layer remained higher even at the 22nd day. The lower layer of soil *B* became more active than the upper on the 15th day; the 12-in. sample of this soil, in other respects also, as shown previously, differs little from the 6-in. sample.

TABLE XII

AMMONIA PRODUCED FROM UREA: SAMPLES FROM DIFFERENT DEPTHS

Soil	Depth, in.	Ammonia nitrogen, mgm. per cc.					
		Days	*3*	*7*	*9*	*15*	*22*
S Aug. 4	0– 6		3.03	25.70	27.38	27.99	26.11
	7–12		0.57	7.37	11.50	20.63	22.90
S Sept. 15	0– 6		5.11	21.23	26.85	27.33	—
	0–12		2.01	15.41	21.03	27.51	—
R	0– 6		2.21	27.80	27.76	—	—
	0–12		1.25	21.66	28.04	—	—
B	0– 6		1.71	11.20	15.24	20.58	22.71
	0–12		0.96	8.08	13.32	24.20	26.80

General Discussion

The microbiological activity of these podsol soils appears to be controlled by the organic-matter relations of the well-differentiated horizons. The biological activity is almost entirely confined to the mat of organic matter that covers the mineral soil. The data show that the activity of the mineral horizons of the soil through which, and to which, the organic and inorganic materials are leached can be expressed as a small fraction only (less than 4%) of the activity of the surface layer of organic matter. Disturbance of the relative positions of the horizons, during the time covered by the analyses reported above, does not appear to cause any beneficial or harmful results in so far as concerns the aerobic bacteria appearing on a standard plate, or the nitrifying bacteria. The results suggest that the reduced activity in the lower layers of soil is due to position and not to the presence of toxic material removed from the organic-matter horizon by leaching and deposited in the B horizon.

The evidence submitted with regard to the plate counts of bacteria on media prepared with the solutions from the three horizons and the tests for the ammonification of urea by equal masses of soil from the separate horizons and different depths, would appear to confirm these conclusions.

It is perhaps worth while to record here that the activity of these virgin soils, as shown by the evolution of carbon dioxide, is of a higher order than the activity of the adjoining land that has been under normal cultivation for many years (see Table XIII). The closer agreement between these values in the case of soil *S* may be due to the fact that this has been cultivated for many years to a greater depth than is customary.

Bacterial numbers, on the other hand, are considerably higher after cultivation. These soils have received organic manures and chemical fertilizers since they were originally broken up; this would no doubt account for the increased number of these organisms.

TABLE XIII

COMPARISONS BETWEEN MICROBIOLOGICAL ACTIVITIES OF VIRGIN AND CULTIVATED SOILS (SAMPLES OF 0–6 IN. DEPTH)

Soil	*S* (Aug. 4)	*R* (Aug. 18)	*B* (July 21)
	Carbon dioxide, mgm. per 100 gm. per 100 hr.		
Virgin	41	52	75
Cultivated	47	32	35
	Bacteria and actinomyces, per gm.		
Virgin	980,000	1,920,000	730,000
Cultivated	10,540,000	10,950,000	14,440,000

Summary

1. Microbiological characteristics of representative virgin soils of the upland podsol group of Quebec are described and discussed.

2. The organic-matter horizon of virgin podsol soils is biologically more active than the leached layer or the horizons of accumulation, as shown by the evolution of carbon dioxide in fresh samples, by nitrification of soil nitrogen, by numbers of bacteria and actinomyces, and by the production of ammonia from urea.

3. The amounts of carbon dioxide evolved and the numbers of bacteria and actinomyces in the leached layers are about 4% of those in the organic-matter horizons in two widely separated soils.

4. The soil solutions from the three horizons studied contain sufficient nutrients for the same number of bacteria appearing on a standard medium.

5. The number of bacteria and the amount of available nutrients in the horizons control to some extent the rate of ammonification of urea in culture solution.

6. Mixture of the organic-matter horizon with soil from the leached layer and from the horizon of accumulation reduces the activities of the microorganisms to a lower level than that shown by the organic-matter horizon. The reduction in bacterial numbers appears to be due to dilution of the larger population.

7. Evidence is submitted that reduced activities are not due to the presence of toxic compounds.

8. The amount of carbon dioxide evolved from samples of virgin soil is greater than that from the soil after normal cultivation, but the numbers of bacteria in the cultivated soils are considerably greater than those in the virgin soils.

Acknowledgments

The authors are indebted to the Department of Chemistry, Macdonald College, for analyzing the samples for carbon and nitrogen, and for the determination of the hydrogen ion concentration of some of the samples, and for facilities for determining loss on ignition.

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THE BINOCULAR OSCILLATION AND FUSION OF COLORS[1]

By John F. Allen[2]

Abstract

When pairs of colors fall upon the same area of a retina it is impossible to separate the receptor actions in the retina from those that take place in the visual centres of the cortex. If the separate colors, however, are allowed to fall upon the two eyes, the fusion color effects must be attributed to the sensations in the cortex.

In this investigation the binocular fusion of complementary colors into white is confirmed, as well as the binocular fusion of red and green colors into yellow. The sensation of yellow is therefore proved to be compounded of the fundamental sensations, red and green.

The binocular oscillation or rivalry of colors was specially studied and shown to be due not to fluctuations of attention, but probably to the oscillation of the neural processes of inhibition and facilitation. The oscillation of colors is thereby connected with other phenomena of vision.

Introduction

In conducting investigations on vision it has been the common practice to make the observations with a single eye, and to disregard completely the influence of the other eye which usually was kept in a state of darkness adaptation. In view of the complicated nature of the sense of vision, this procedure involves many uncertainties since there is no way of separating effects which are purely retinal from those which pertain to the nervous tracts and the cortical centres. An example of this uncertainty is found in the phenomenon of contrast, which has been attributed by Helmholtz to psychological action in the cortex, and by Hering and by Mrs. Ladd Franklin to the action of light on photochemical substances in the retina. Uniocular investigations have also suggested the idea that the production of white by complementary colors results from similar retinal reactions. Uniocular experimentation at best involves the action of one-half of the visual apparatus, whereas the mechanism of vision is so constituted that perfect action can be obtained only binocularly.

In binocular investigations the cortical actions, in part at least, can be separated from those which occur in the retina. The effects of color mixture can therefore be attributed to the visual centres in the cortex where color sensations are elaborated. Hence it is rather surprising to find that so little quantitative work has been done on binocular color mixtures, and on binocular phenomena generally.

From the experimental standpoint there is perhaps some reason for the paucity of measurements in this field. In binocular researches the spectroscopic apparatus must necessarily be much more elaborate than that needed for uniocular investigations. While binocular fusion of two color fields can

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be accomplished by suitable methods with great ease and with no perceptible strain upon the eyes, the observations involve color rivalries or oscillatory effects which at times render measurements difficult.

For a long time the possibility of binocular fusion of colors was a subject of much controversy (9, vol. 3, p. 505). Dove, Regnault, Brücke, Ludwig, Panum and Hering, among the earlier observers, strongly affirmed the occurrence of fusion, while Meyer, Volkmann, Meissner and Helmholtz were equally positive in asserting the opposite view. The existence of binocular fusion of colors has now ceased to be a matter of dispute, as numerous methods of observation with the aid of colored glasses, papers and metal disks, as well as the spectrum, have been devised to illustrate the phenomena. Schenck (11), Stirling (12), Dawson (4, 5), Hecht (8) and von Kries* have described qualitative methods for obtaining fusion, though von Kries confesses that only with great difficulty did he succeed in obtaining unmistakable results. Several of these observers found that the presence of contours or patterns greatly facilitated the observations. Foucault and Regnault (7), as well as Dove (6), obtained binocular fusion by simultaneously viewing through separate telescopes two colors of a spectrum projected upon a screen. Trendelenburg (13, 14), however, was the first to make accurate comparative measurements of uniocular and binocular fusion of complementary spectral colors to form white, and of red and green colors to produce yellow. The measurements of complementary colors were amply confirmed and extended by Rochat (10). Rochat, as well as Trendelenburg, noticed that the binocular fusion of complementary colors follows the same rules that govern uniocular fusion, but that the intensities of the colors of shorter wave-lengths are smaller for binocular than for uniocular mixtures in the less refrangible portion of the spectrum as far as the wave-length 480 $m\mu$. The intensities of this color were the same for both types of fusion, but for still shorter wave-lengths the binocular intensities became the greater. The reason for this change at the wave-length 480 $m\mu$ is not apparent. But it may be remarked that F. Allen (1) found this hue to be an equilibrium color which consequently does not influence inductively its complementary sensations.

These facts are clearly brought out in Tables I and II, which are reproduced from the papers of Rochat and Trendelenburg. The wave-lengths of the component complementary colors are within brackets, and the proportional intensities, originally measured in hundredths of a centimetre of slit width, are given by the numbers before the brackets. The column of "Proportion" on the right of the tables gives the ratios of the uniocular to the binocular intensities of the shorter wave-lengths. The ratio 3 : 2.2, for example, is equivalent to 1.37 : 1.

It is worthy of note, especially in Table II, that the intensities of the shorter wave-lengths from 498 $m\mu$ to 491 $m\mu$ are conspicuously greater than those of the longer and shorter wave-lengths 502 $m\mu$ and 449 $m\mu$. If the fundamental sensation curves of König and of Abney in Figs. 1 and 2 be

*See Ref. 9, Vol. 3, p. 529.

TABLE I

INTENSITY OF COMPLEMENTARY COLORS (ROCHAT)

Uniocular	Binocular	Proportion
1 (671 $m\mu$) + 3 (493 $m\mu$) = White	= 1 (671 $m\mu$) + 2.2 (493 $m\mu$)	1.37 : 1
1 (656 $m\mu$) + 5.7 (492 $m\mu$) = White	= 1 (656 $m\mu$) + 4.3 (492 $m\mu$)	1.34 : 1
1 (617 $m\mu$) + 6 (489 $m\mu$) = White	= 1 (617 $m\mu$) + 5.4 (489 $m\mu$)	1.1 : 1
1 (585 $m\mu$) + 11.5 (485 $m\mu$) = White	= 1 (585 $m\mu$) + 11.8 (485 $m\mu$)	0.97 : 1

TABLE II

INTENSITY OF COMPLEMENTARY COLORS (TRENDELENBURG)

Uniocular	Binocular	Proportion
1 (671 $m\mu$) + 5.5 (502 $m\mu$) = White	= 1 (671 $m\mu$) + 2.05 (502 $m\mu$)	2.7 : 1
1 (616 $m\mu$) + 23.7 (498 $m\mu$) = White	= 1 (616 $m\mu$) + 11.8 (498 $m\mu$)	2 : 1
1 (600 $m\mu$) + 22.8 (495 $m\mu$) = White	= 1 (600 $m\mu$) + 13.5 (495 $m\mu$)	1.7 : 1
1 (589 $m\mu$) + 17.1 (491 $m\mu$) = White	= 1 (589 $m\mu$) + 13.55 (491 $m\mu$)	1.3 : 1
1 (570 $m\mu$) + 8.7 (449 $m\mu$) = White	= 1 (570 $m\mu$) + 17.3 (449 $m\mu$)	0.5 : 1

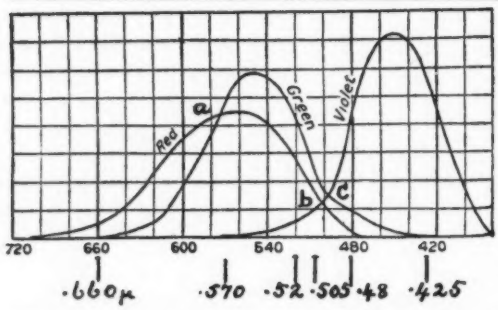

FIG. 1. *Normal sensation curves (König). The six colors specially marked are equilibrium colors. (From Parsons, J. H. Colour vision, 2nd ed. Cambridge Univ. press, 1924, p. 235.)*

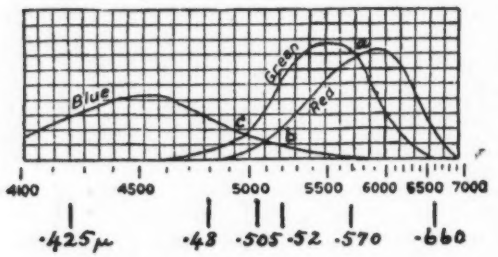

FIG. 2. *Normal sensation curves (Abney and Watson). The six colors specially marked are equilibrium colors. (From Parsons, J. H. Colour vision, 2nd ed. Cambridge Univ. press, 1924, p. 260.)*

examined, it will be seen that the group of wave-lengths from 498 $m\mu$ to 491 $m\mu$ fall at the lowest parts of the curves, whereas their complementary colors, 616 $m\mu$ to 589 $m\mu$, correspond to the much greater ordinates of the red and green curves. As the sensation of white is due to equal stimulation of all three primary sensations, red, green and violet, it is evident that extra large intensities of the shorter wave-lengths in question will be required to balance the excitation of the longer wave-lengths. But when the short-wave components are greater than 498 $m\mu$ and less than 491 $m\mu$, their ordinates in the sensation curves increase in length, which indicates that the disparity between the intensities of

the complementary components becomes less than before, and therefore less intense stimulation by the shorter wave-lengths is required to balance the excitation by the longer.

This explanation applies to all the binocular intensities except the last value 17.3 in Table II, which, however, is the mean of only two measurements 13.6 and 21, the former of which is the same as for the wave-length 491 $m\mu$. The enhancing inductive action of stimulation of one eye upon the sensitivity of the other is probably sufficient to account for the smaller values of the binocular intensities than of the uniocular.

During the course of the present investigation, two sets of binocular complementary colors were obtained by the writer and W. A. Allen. The sets differ from each other and also from those obtained by Helmholtz for uniocuiar observation, but not by greater amounts than those which are generally found between estimates of different observers. In Table III the first and fourth columns contain the pairs of complementary colors according to Helmholtz (9, vol. 2, p. 126). The wave-lengths in the first column were used also by the writer and his assistant for observation with the left eye, while the second and third columns contain respectively the binocular complementaries obtained with the right eye by himself and by W. A. Allen.

TABLE III

COMPLEMENTARY COLORS

Helmholtz. Also used by J.F.A. and by W.A.A. for observation by left eye. Wave-length, $m\mu$	Right eye, J.F.A. Binocular Wave-length, $m\mu$	Right eye, W.A.A. Binocular Wave-length, $m\mu$	Helmholtz Uniocular Wave-length, $m\mu$
656.2	500.5		492.1
607.7	490		489.7
585.3	476.3		485.4
492.1	613	630	656.2
489.7	597	599.3	607.7
485.4	592	592.3	585.3
482.1	589.5	584.2	573.9
464.5	583.3	580.8	567.1
461.8	578	572	564.4

The Binocular Production of Yellow

In addition to his observations on complementary colors, Trendelenburg also measured the uniocular and binocular intensities of the red and green hues required to form the sensation of yellow, as in the Rayleigh equation, 671 $m\mu$ + 535 $m\mu$ = 589 $m\mu$. It was also found that the uniocular intensity of the shorter wave-length, green, was always greater than the binocular in the ratio of from 4 : 1 to 14 : 1, according to the observer.

F. Allen has suggested that two of the three fundamental color sensations correspond to the wave-lengths 687 $m\mu$ (red) and 530 $m\mu$ (green), for the reason that these colors together with the third primary, probably 410 $m\mu$, elicited conspicuously large inductive effects of depression and enhancement of retinal sensitivity. The color 550 $m\mu$, however, excited inductive effects nearly if not quite equal in magnitude to those of 530 $m\mu$, so that it was uncertain as to which hue of green precisely was the fundamental. There seemed to be no way of deciding between them. It is evident, however, that these two fundamental colors should fuse binocularly into yellow. The mixture 687 $m\mu$ and 530 $m\mu$ was tried, but the fusion yellow was greenish in hue. When the wave-length 550 $m\mu$ was substituted for 530 $m\mu$, the fusion showed no tinge of the component colors and differed from spectral yellow only in saturation. This hue of green therefore probably represents very closely the fundamental green sensation. While the colors of the Rayleigh equation fuse equally well into yellow, yet the wave-length 671 $m\mu$ is not as pure a red as 687 $m\mu$, nor does it produce such pronounced inductive reactions. The latter wave-length is therefore to be preferred as the fundamental red color.

The production of a yellow sensation by the binocular fusion of red and green colors throws considerable light upon one of the most important questions of color vision, that is, whether yellow is a simple or a compound sensation. The uncertainty regarding this question has been one of the chief reasons for the numerous theories of color vision, many of which are based on the assumption that yellow is quite as unique a sensation as red and green. It has been known from the time of Newton that uniocular fusion of red and green colors gives a yellow sensation. This experiment has been considered to be indeterminate since the component colors might be regarded as uniting to form white, while the yellow color, assumed to be evoked in addition by both red and green, appeared of the same hue as spectral yellow but less saturated. When the fusion of red and green is made binocularly, however, no question of such retinal actions can possibly arise, and the formation of yellow must therefore be attributed to the fusion of a pair of primary sensations in the cortex itself.

By these exact and conclusive spectroscopic experiments of Trendelenburg, confirmed qualitatively by other investigators such as von Kries, and now by the writer, the yellow sensation has been unquestionably proved to be compounded of the primary sensations red and green. Theories of color vision which are based on the assumed primary character of the yellow sensation consequently become untenable.

Binocular Oscillation of Colors

The phenomena of binocular oscillation appear when different colors are simultaneously viewed by each eye, and they are characterized by the successive presentation in consciousness of one color or the other, with the color

resulting from their fusion occasionally intervening. When, for example, red and green are the colors observed, there are seen in succession red, occasionally yellow resulting from fusion, green, and so on repeatedly.

The phenomena of binocular rivalry, or oscillation as it is preferable to term it, were originally attributed by Helmholtz, with the subsequent concurrence of other investigators, to the shifting of attention of the observer, an explanation arising no doubt from his custom of referring many phenomena, such as those of simultaneous contrast, to psychological action, when no satisfactory physiological principles were known with which they could be related. While the writer was observing the binocular fusion of complementary colors, the periodic oscillation of several of the pairs was so pronounced that it was decided to make time measurements in order to determine what degree of regularity existed in the oscillations and so test this application of the theory of attention.

There happened to be in the laboratory of F. Allen two similar Hilger constant deviation spectrometers mounted upon narrow bases but with their collimators turned in opposite directions. The telescopes were placed nearly parallel to each other as in Fig. 3, but separated by the distance between the eyes of the observer, so that each eye independently viewed a different but similar spectrum. After a little practice with the accommodation of the eyes, the colors could easily be fused with no perceptible effort, and the periods of oscillation measured. Illumination was provided by two 400-watt lamps, one in front of each collimator slit. A third collimator was used to direct a beam of white light by reflection into the telescope for the left eye, where it appeared just above the color patches in the field of view. The white light was used for comparison during the observations on binocular complementary fusion. A single patch of white light, however, is of very little value. For if white light falls upon one eye while the other has only a dark field in front of it, the fusion of the two gives an unsatisfactory gray field which is useless for purposes of comparison. The intensity of each color was controlled by the width of the slit, and the sizes of the patches of light were regulated by shutters both at the slits and in the eyepieces.

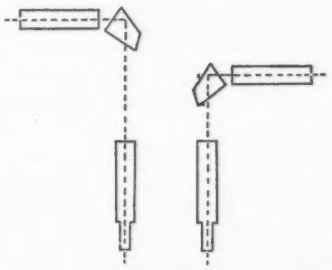

FIG. 3. *Arrangement of two spectrometers for observations of binocular fusion and oscillation of colors.*

For measuring the periods of oscillation a rotating drum chronograph was used, on which a timing pen, operated electromagnetically by a clock, marked off half-second intervals. Close beside the time recorder, a second pen was attached to an arm about 20 cm. long which was pivoted in the middle and adjusted so that its excursions ranged about a centimetre on each side of the time tracing. This pen was manipulated by the observer. While watching the binocular oscillation of colors, the observer swung the pen

above or below the line when the patch of light changed from one color to the other. Two such graphs are shown in Figs. 4 and 5. In all investigations on the senses, a period of training is always necessary in order to secure reliable and consistent measurements of any kind. Therefore in recording the periods of color oscillation considerable practice is necessary. The great difficulty in the present research lay in inducing the hand to shift the tracing pen at the instant a change of color occurred. No warning is given to prepare the mind for the change. One simply becomes aware that a different color is in view. In spite of this difficulty, quite consistent results were finally obtained for several pairs of colors as well as for a single pair at different intensities.

A third pen, set immediately above the tracing pen, was sometimes found useful. It was operated electromagnetically by an assistant whose duty it was to watch the eyes of the observer and record on the drum the moments of winking. In this way the connection of winking with the rate and nature of the oscillation was ascertained.

Results of Observations

In Fig. 4 is shown a typical chronographic record of the binocular oscillation of the colors red, 687 $m\mu$, and blue, 480 $m\mu$. The graph reads from left to right. The horizontal line of dots, A, records the time, the distances between successive dots representing half-second intervals. The full line represents the observed oscillations from one color to the other, the parts above the horizontal time line representing the red phases, and the parts below the horizontal, the blue phases of the oscillations. The three places where the full line breaks at the time line represent intervals of fusion. The dots above the oscillating line represent moments when winking occurred. There is evidence from this graph that fusion of colors is generally only an occasional episode in the more regular oscillations.

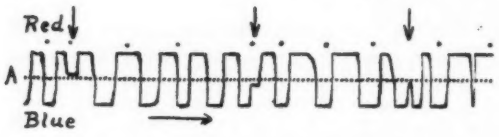

FIG. 4. *Graph showing periodic binocular oscillation and fusion of red and blue colors. Horizontal line of dots, A, indicates half-second intervals. Upper line of dots indicates moments of winking. Vertical arrows indicate fusion of colors.*

Fairly regular winking is a natural process to which the eyes are accustomed and to which the actions concerned in vision are adapted. It is therefore to be expected that when winking is inhibited the effort required for that purpose will influence visual processes in some degree. The graph shown in Fig 5, which was obtained with the same colors and under the same conditions

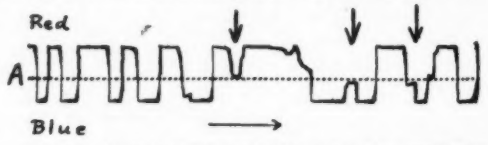

FIG. 5. *Graph showing periodic binocular oscillation and fusion of red and blue colors. Horizontal line of dots, A, indicates half-second intervals. Vertical arrows indicate fusion of colors.*

as for Fig. 4, except for the absence of winking, shows much greater irregularity than the previous figure. The irregularity increases with the time during which winking is restrained.

In Tables IV and V are shown the periods of time during which the red color, 687 $m\mu$, in the right eye, and the blue color, 480 $m\mu$, in the left eye, were visible, as well as the occasional periods during which fusion occurred. Both sets of times were obtained by the writer with colors of fairly high intensities. No figure corresponding to Table IV is given, but the data in Table V are obtained from the graph in Fig.5. These measurements are

TABLE IV

PERIODS OF OSCILLATION AND OF FUSION (J.F.A.). WINKING

Red, 687 $m\mu$ in right eye, sec.	1.50	1.00	1.25	1.50	0.75	1.25	1.75	1.25	2.00	1.50	2.00	2.25	1.25	1.25	1.25	1.50	Av. 1.45
Blue, 480 $m\mu$ in left eye, sec.	1.50	2.00	2.25	2.00	1.00	1.50	1.50	2.50	1.00	1.50	1.50	1.25	3.00				1.73
Fusion, sec.	0.75	1.25	0.75	1.25	1.00	0.25	3.00	0.50	2.50								1.25

TABLE V

PERIODS OF OSCILLATION AND OF FUSION (J.F.A.). NON-WINKING (SEE FIG. 5)

										Av.
Red, 687 $m\mu$ in right eye, sec.	1.25	1.25	2.50	1.25	2.00	1.35	4.50	2.50	2.00	2.07
Blue, 480 $m\mu$ in left eye, sec.	1.25	1.50	1.00	1.75	2.00	3.00	1.50	1.00	2.00	1.66
Fusion, sec.	0.75	0.75	1.25	1.00	0.75	0.50				0.83

illustrative of the variation in magnitude of consecutive periods. Since in the absence of precise knowledge of what is happening in the eyes, the individual periods are of little importance, in Table VI only average periods are given.

By comparing the values for the two observers in Table VI it will be noticed that quite generally the periodic times for the red and blue colors are reversed. Where W.A.A. finds the periods for red longer than for the blue, J.F.A. finds the opposite to hold and the periods for blue are longer than for red. In the case of both observers, however, when the colors in the eyes were interchanged, the periodic times of oscillation were also reversed.

It was formerly noticed by F. Allen (2) that in a few minutes after any sense organ had been stimulated, it acquired an enhanced state of excitability for subsequent stimulation. In Section 2 of Table VI, repetition of oscillation measurements after a rest of ten minutes showed diminished periods for both red and blue phases. These reduced times may therefore be attributed to the enhanced activity of the receptors. While the periods of oscillations were shorter, the fusion periods, in the case of W.A.A., were doubled.

TABLE VI

PERIODS OF OSCILLATION AND FUSION FOR W.A.A. AND J.F.A.†

1. Right eye, red, 687 $m\mu$; left eye, blue, 480 $m\mu$

	Both colors high intensity	Same, non-winking	Both colors low intensity	Intensities equalized	Red, low intensity; blue, high intensity	Red, high intensity; blue, low intensity
W.A.A.						
Mean period for 687 $m\mu$, sec.	1.57	2.00	1.69	2.17	1.61	1.89
Mean period for 480 $m\mu$, sec.	1.68	1.92	1.58	2.08	2.11	1.65
Mean period for fusion, sec.	1.08	1.50	0.87	1.33	1.29	1.16
J.F.A.						
Mean period for 687 $m\mu$, sec.		*1.56	1.59	1.38	1.15	1.50
Mean period for 480 $m\mu$, sec.		*2.25	1.70	1.77	1.73	1.46
Mean period for fusion, sec.		*1.50	None	1.13	1.04	1.15

2. Colors interchanged. Right eye, blue, 480 $m\mu$; left, red, 687 $m\mu$

	Low intensities		Repeated after 10 min. rest	
	W.A.A.	J.F.A.	W.A.A.	J.F.A.
Mean period for 687 $m\mu$, sec.	1.62	1.77	1.20	1.57
Mean period for 480 $m\mu$, sec	1.79	1.58	1.56	1.40
Mean period for fusion, sec.	0.65	None	1.25	None

3. Other pairs of colors. Right eye, red, 687 $m\mu$; left eye, blue, 450 $m\mu$, and green, 550 $m\mu$. Intensities equalized

	W.A.A.		J.F.A.	
Mean period for 687 $m\mu$, sec.	1.88	0.97	1.43	1.13
Mean period for 450 $m\mu$, sec.	1.69		1.86	
Mean period for 550 $m\mu$, sec.		2.18		2.00
Mean period for fusion, sec.	1.36	1.25	1.03	1.00

† *All observations were made with winking at normal rate, except those marked "non-winking".*

* *In these measurements for J.F.A. the red was of low intensity and the blue of high intensity*

Both observers also found that when green, 550 $m\mu$, was one of the oscillating colors, its influence was always predominant. This fact is given numerical verification in Section 3, Table VI, where the green phase lasted a noticeably longer time than the red though the intensities of the colors were equalized.

It was discovered by Professor F. Allen (1) that six colors of the spectrum, 660 $m\mu$, 572 $m\mu$, 520 $m\mu$, 505 $m\mu$, 480 $m\mu$ and 425 $m\mu$, were incapable of either enhancing or depressing the sensitivity of the visual receptors. For this reason they were called equilibrium colors. Their peculiar character

was first noticed when they were used at high intensities. Whether in all cases they retain their equilibrium character at lower intensities as well, is open to question. From experimental evidence, however, it is quite certain that the color 505 $m\mu$ does possess an equilibrium character from the lowest to the highest range of intensities; and the same is probably true for some wave-length close to 570 $m\mu$ or 572 $m\mu$. For theoretical reasons which will shortly be discussed it seemed advisable to examine pairs of these peculiar colors for their powers of oscillation. For since they have an equilibrium nature they should not oscillate with each other at all. The results of this examination are given in Table VII. The second and third pairs of colors are not equilibrium colors. Where no oscillation occurred steady fusion of colors took place.

TABLE VII

BINOCULAR OSCILLATION AND FUSION OF COLORS

Pairs of colors		Oscillation at low intensity	Oscillation at high intensity	Pairs of colors		Oscillation at low intensity	Oscillation at high intensity
$m\mu$	$m\mu$			$m\mu$	$m\mu$		
570	660	Slight	Slight	480	570	Slight	Some
535	687	Decided	Decided	480	520	None	None
535	671	Decided	Decided	425	660	Some	Some
520	660	Some	Some	425	570	Slight	None
505	671	Some	Some	425	520	None	None
505	570	None	None	425	505	None	None
480	660	Some	Some	425	480	None	None

From Table VII it will be noticed that the equilibrium colors, 425 $m\mu$, 480 $m\mu$, 505 $m\mu$, and 520 $m\mu$, in any combinations that were tried, gave no indication of binocular oscillation. Fusion of the two colors was always maintained for any length of time of observation. When 425 $m\mu$ was used with 570 $m\mu$, slight oscillations were observed at low intensities, but complete and stable fusion occurred at high intensities. Special attention may be directed to the fact that the combination 505 $m\mu$ and 570 $m\mu$ gave not the slightest indication of oscillation, but perfect fusion was maintained at all intensities. The color 480 $m\mu$ with 570 $m\mu$ was found to give at low intensities slight evidence of oscillation which was more pronounced when the intensities were much increased. The combination 570 $m\mu$ and 660 $m\mu$ gave only slight oscillations at all intensities. The equilibrium band of color at 660 $m\mu$ is so narrow that it requires a two-prism spectrometer to disperse the spectrum sufficiently to obtain it. In these experiments single prisms were used so that the color in question could not be obtained sufficiently pure to be free from oscillatory effects. Probably a more complete study of these oscillatory phenomena would enable the exact equilibrium hues to be determined.

Theoretical Considerations

The usual name given to the phenomena of oscillation is color rivalry. Many peculiarities of its operation have been observed and descriptions of them may be found in the treatises on color vision. Obviously the term "rivalry" itself affords no suggestion of an explanation of the alternately successful competition of the colors for predominance in consciousness. Taken by itself the term "oscillation" is open to the same objection unless some condition can be found which does the oscillating of which the color observed is merely the index.

As mentioned above, Helmholtz ascribed the phenomena to fluctuations of attention. The periodic nature of the observations which have been described, the reversal of periodic times with the interchange of colors in the eyes, and the change of periodic times with intensities, are all opposed to that theory. But the theory of attention as an explanation of rivalry is rendered untenable by the fact that many combinations of equilibrium colors fail completely to give any oscillatory effects. This is particularly true of the colors, yellow 570 $m\mu$, and green 505 $m\mu$, which are the most complete equilibrium colors known. They are very dissimilar in appearance and the first is the brightest color in the spectrum. In addition to this evidence against the theory of attention, the writer found all attempts to hasten or delay the oscillations of colors by arbitrary fixation of attention to be futile; they were beyond voluntary observation or control.

Two neural processes, inhibition and facilitation, are well known in physiology, and these have been found by F. Allen (3) to be associated with all visual and indeed with all sensory activities. Their combined function is to control the sensitivity of the receptor apparatus, inhibition to depress and facilitation to enhance it. Every color is a physiological stimulus with individual characteristics, especially in regard to its power of eliciting these two inductive or sensory reflex processes. When the stimulating power of the color results in a perfect balance between them, the color has an equilibrium character. It is quite possible for a color to have this power at high intensities and not at low, or to possess it at low but not at high intensities of stimulation. When the color falls upon the retina of one eye the inductive processes, whether unbalanced or in equilibrium, control the sensitivity of the other retina. If when two colors fall upon the two eyes, the inductive processes exactly balance each other, they may be continually fused into a stable compound color with neither component able to predominate over the other. But when the inductive actions are unbalanced, as generally happens, the inhibitory process will then depress the reception of one color and allow the other to appear exclusively in consciousness, until the inductive actions become reversed and the first color in its turn becomes visible. Occasionally the inductive actions may balance each other for a second or two with the result that fusion of the two colors occurs, after which the oscillation again becomes evident.

If, when fusion of the two colors occurs, the three fundamental sensations at the same time are equally stimulated, the compound or fusion sensation is necessarily white. The binocular fusion of pairs of complementary colors is therefore precisely the same in nature as fusion in general, except that it always conforms to the special condition of equality of stimulation of all three sensations. When, however, equality of binocular stimulation is confined to the two fundamental sensations, red and green, the fusion product is not white but yellow.

The phenomena of binocular oscillation and fusion of colors in general, of binocular fusion of complementary and of equilibrium colors, and of the binocular production of yellow, are therefore indicated to be but different manifestations of the inductive neural processes inseparably associated with the action of light of different wave-lengths upon the eyes, combined with the equal or unequal stimulation of the three primary sensations red, green and violet, or with equal stimulation of only the red and green sensations.

Acknowledgment

The author desires to express his thanks to Professor Frank Allen, under whose direction the investigation was undertaken, for supplying the necessary experimental facilities.

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THE VISCOSITIES OF ACETALDEHYDE AND PARALDEHYDE[1]

By W. H. Hatcher[2] and C. T. Mason[3]

Abstract

The viscosities of acetaldehyde, of paraldehyde and of mixtures of these are given at 15° C., using materials freshly distilled out of contact with oxygen.

Introduction

The necessity for viscosity data on acetaldehyde, paraldehyde and mixtures of these two required the determination of such values. This involved the preparation of these aldehydes and their use out of contact with oxygen. Results previously obtained (1) provided specific gravity data for these aldehydes and their mixtures. Also some unpublished work indicated that contamination of acetaldehyde with oxygen is significant only in the gaseous phase.

Preparation of Reagents

A high grade of paraldehyde was distilled fresh every day, and the fraction between 123° and 124° C. used. The same quality of paraldehyde was used with sulphuric acid for distillation of acetaldehyde, and this was redistilled, both processes being carried on in an atmosphere of nitrogen. The acetaldehyde was also freshly distilled each day.

Apparatus

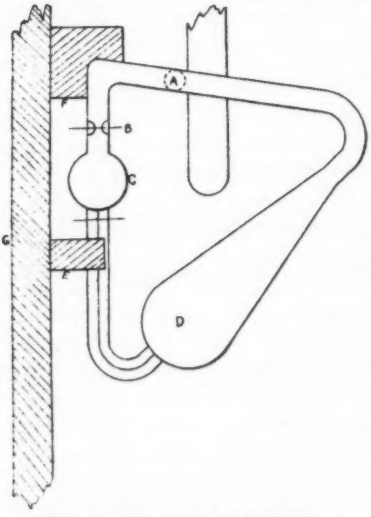

Fig. 1. *Diagram of viscosimeter.*

After several trials an apparatus (Fig. 1) was devised on the principle of the Ostwald viscosimeter. It is roughly triangular in shape, having at *A* a side arm attached at right angles to the plane shown; through this side arm were admitted the liquids studied. Particularly, however, this arm served as a pivot about which the body of the viscosimeter could be rotated within the limits of the thermostat. *G* represents the side of the thermostat; *E* and *F* rests against which the vertical flow arm was held during an experiment. After filling at *A*, partial rotation filled this flow-arm to the same height for successive measurements, so that passage of the liquid past *B* and through *C* would be uniform. Thus the apparatus with the appropriate volume of liquid stoppered within could be kept in the same condition for each reading without opening.

[1] *Manuscript received February 17, 1933.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada.
[2] *Associate Professor of Chemistry, McGill University.*
[3] *Graduate student in Chemistry, McGill University.*

Experimental

Calibration of the viscosimeter was effected with boiled, distilled water. Where mixtures of the aldehydes were used, mixing was previously carried out by weighing, the appropriate volume being later pipetted in. These operations which involved aldehydes were carried out in an atmosphere of pure nitrogen. Although some contact with air was at times unavoidable, yet no aldehyde vapor which had come into contact with oxygen was able to get into the viscosimeter. Extreme precautions as to cleanliness were observed throughout. The temperature maintained throughout was 15° C., the bath variation being ± 0.1° C. at the most.

Results

The results which follow were obtained using a constant liquid volume of 20 cc. at 15° C., and are the mean of times of flow which agreed to within 0.5 sec. Calibration with water showed a time of flow of 338.2 sec.

The results for the pure aldehydes and their mixtures are included in Table I. For purposes of comparison the composition of the mixtures (by weight) was plotted on a large scale against time of flow; from this the times of flow were read off at 10% intervals. Using these values and the specific gravities at these percentages from the curve of Hatcher and Kay (1), the specific viscosities compared with water at 15° C. were calculated.

If the values shown in Table I be represented graphically a smooth curve is obtained lying (for the mixtures) always below the ideal joining acetaldehyde and paraldehyde, the maximum deviation occurring at 50% concentrations, where the actual specific viscosity is 63% of the ideal.

TABLE I

VISCOSITIES OF ACETALDEHYDE-PARALDEHYDE MIXTURES

Percentage acetaldehyde	Sp. gr.	Time of flow, sec.	Specific viscosity at 15° C.	Percentage acetaldehyde	Sp. gr.	Time of flow, sec.	Specific viscosity at 15° C.
0.0	0.9963	403	1.188	60.0	0.8650	141	0.3610
10.0	.9750	290	0.8368	70.0	.8448	126	0.3150
20.0	.9515	238	0.6702	80.0	.8250	113	0.2759
30.0	.9295	203	0.5584	90.0	.8057	101	0.2408
40.0	.9080	178	0.4783	100.0	.7865	92.3	0.2147
50.0	.8862	158	0.4144				

Using the value 0.1144 as the absolute viscosity of water (2, p. 10), the latter for paraldehyde becomes 0.1359 and for acetaldehyde 0.02456. This last compares with 0.02321 as found by Thorpe and Rodger (3). No value for paraldehyde was known previously.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

V. THE CONSTITUTION OF ADLUMINE[1]

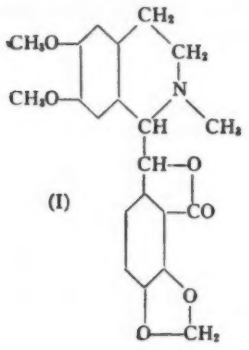
By Richard H. F. Manske[2]

Abstract

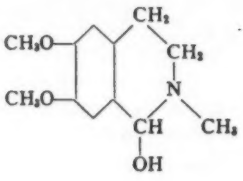
A chemical examination of the alkaloid *adlumine*, which the author recently isolated from *Adlumia fungosa*, has disclosed an intimate relation to hydrastine and bicuculline. It differs from hydrastine only by the fact that the positions of the methylene-dioxy and the dimethoxy groups are reversed. Hydrolytic oxidation yields the expected products.

During an investigation dealing with the alkaloids of *Adlumia fungosa* (2) the author isolated an alkaloid having the empirical formula $C_{21}H_{21}O_6N$, to which the name *adlumine* was given. A methoxyl determination disclosed the presence of two such groups and the nitrogen appeared to be tertiary.

Since the alkaloid is isomeric with hydrastine a close relationship was suspected and this was further confirmed by the observation that oxidation with manganese dioxide in dilute sulphuric acid yielded a bluish fluorescent solution. If it be assumed that the nuclear substituent oxygen atoms occupy the same positions in this alkaloid as they do in hydrastine and in bicuculline the only possible formula for adlumine is (I).



(I)



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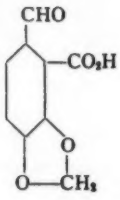

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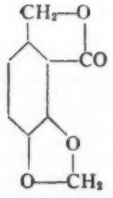

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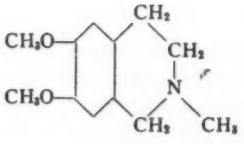

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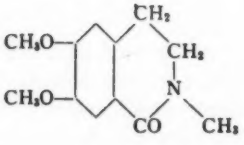

(VI)

[1] *Manuscript received March 3, 1933.*
Contribution from the National Research Laboratories, Ottawa, Canada.

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On this basis hydrolytic oxidation should yield 4, 5-dimethoxy-2 (β-methylaminoethyl)-benzaldehyde (II) and 2-carboxy-3, 4-methylenedioxy-benzaldehyde (III). Experimentally this supposition was readily verified. The aminoaldehyde (II) was first prepared from laudanosine by Pyman (3) and one is grateful to this author not only for the thoroughness with which it was characterized but also for the fact that all melting points were corrected. In the present case the free base (m.p., 123-124° C.)* was characterized by conversion to the picrate (m.p., 170° C.), and more specifically by the production from it, by treatment with alkali (Cannizzaro), of a mixture of 1, 2, 3, 4-tetrahydro-2-methyl-6, 7-dimethoxy-isoquinoline (V) (m.p., 84° C.) and the 1-keto-derivative (VI) (m.p., 126° C.). In all cases the properties of the products were those recorded by Pyman.

The acidic fragment was not isolated as such, but reduced at once with sodium amalgam and it then yielded 3, 4-methylenedioxy-phthalide (IV) which proved to be identical with a specimen similarly prepared from bicuculline (2).

Adlumine is thus the third representative of four possible alkaloids of closely related structure with the nuclear oxygen atoms in the same position. The existence of the final member in nature, namely, a tetramethoxy compound, is therefore highly probable, since it is evident that the phytochemical processes are capable of sufficient variation, because of the fact that methoxyl groups have been shown to occur in the two pairs of positions.

Experimental

Hydrolytic Oxidation of Adlumine—

Isolation of the Amino-aldehyde (II)

One gram of the alkaloid was dissolved in a mixture of 2 cc. of concentrated nitric acid and 8 cc. of water and heated on a steam bath for 20 min. The mixture was thoroughly cooled, rendered strongly alkaline with 50% aqueous potassium hydroxide and the liberated base extracted with several successive portions of ether. The combined extract was dried over sodium sulphate, the greater part of the solvent removed and the residual solution cooled. The crystalline base was recrystallized once from ether and melted at 123-124° C.

The *picrate* was recrystallized from methanol by the cautious addition of ether, and melted sharply at 170° C.

The total amount of base obtained from two grams of the alkaloid was heated for 30 min. on a steam bath with an excess of methanolic potassium hydroxide and the tetrahydro-isoquinoline (V) and its 1-keto-derivative (VI) separated and purified by Pyman's method. The former was obtained in fine needles melting at 84° C. and the latter in the form of large flat plates melting at 126° C.

* *All melting points are corrected.*

Isolation of 2-Carboxy-3:4-methylenedioxy-benzaldehyde and its Conversion to the Phthalide (IV)

The alkaline solution from which the aminoaldehyde (II) had been extracted with ether was acidified with sulphuric acid and exhausted with ether. The combined extracts were washed with several small successive portions of water and most of the ether distilled on a steam bath. The remaining solvent was removed *in vacuo* and the solid crystalline residue reduced with sodium amalgam in dilute sulphuric acid solution. The aqueous solution was then extracted with ether, the solvent removed, and the residue recrystallized from hot water with the aid of charcoal. As thus obtained the 3, 4-methylenedioxyphthalide (IV) consisted of colorless elongated needles which melted alone or admixed with a specimen obtained from bicuculline at 232° C.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

VI. *Corydalis sempervirens* (L.) PERS.[1]

By Richard H. F. Manske[2]

Abstract

Corydalis sempervirens has yielded six alkaloids, two of which, bicuculline and bicucine, had been previously found in *Dicentra cucullaria*. The similarity of these two plants is further demonstrated by the presence in the former of cryptopine and, as in the case of other fumaraceous plants, protopine. A new alkaloid, $C_{19}H_{17}O_6N$(?), which has been named *capnoidine* appears to be isomeric with adlumidine but is not identical with it. A very small amount of an unidentified base melting at 201° C. was also obtained.

Although a number of Asiatic species of *Corydalis* have been very thoroughly investigated in regard to the alkaloids present, this is not true of species native to America. The only record of such an investigation is a cursory one by Heyl (1) dealing with *C. aurea*, Willd.

In view of the fact that, as recorded in earlier papers of this series, a number of new alkaloids have been isolated from American species of *Dicentra* it seemed particularly desirable to examine some American *Corydalis* species, and the present communication deals with *C. sempervirens* (L.) Pers. (*C. glauca*, Pursh.). Some difficulty was experienced in obtaining sufficient material for this investigation, so that the record is to be regarded as not quite complete and it is proposed to continue the investigation when more material becomes available.

The total quantity of alkaloid found in this plant was exceptionally small, but this is probably due to the fact that it had reached an advanced state of maturity at the time of collection. Nevertheless, six alkaloids have been isolated in a state of purity. Protopine, which seems to be a constant constituent of all fumaraceous plants, was found in quantity. Two of the other alkaloids were identical with the bases (α and β) previously isolated from *Dicentra cucullaria* (2). Alkaloid (α), which has proved to be new and whose constitution has been elucidated, has recently been named *bicuculline* (3). Alkaloid (β) has now been more completely characterized and since it also appears to be new, the name *bicucine* is proposed for it. Some difficulty has been experienced in obtaining satisfactory carbon analyses, but it is highly probable that the formula $C_{20}H_{19}O_7N$ is the correct one. In this eventuality it may bear the same relation to bicuculline that nor-narceine bears to narcotine.

A fourth alkaloid, which also may belong to this class of bases, was obtained in minute amounts. Analyses indicate the formula $C_{19}H_{17}O_6N$ (or $C_{19}H_{15}O_6N$). This is isomeric with the alkaloid adlumidine which the author has isolated from *Adlumia fungosa* (4). Furthermore, both alkaloids melt at 235°*, but when admixed the melting point is depressed to 210°,

[1] *Manuscript received March 4, 1933.*
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* *Melting points are corrected.*

sintering taking place at 206° C. It is proposed to coin the name *capnoidine* for this base, the word being derived from the term Capnoides, which has been used by some botanists for the genus *Corydalis*.

It may be of interest to call attention to the close relation which the alkaloids of *C. sempervirens* bear to those of *D. cucullaria*. Not only do protopine, bicuculline and bicucine occur in both plants, but the similarity extends to the presence in both species of cryptopine which until recently had been found only in opium. In this connection it is relevant to point out that the alkaloid previously isolated from *D. cucullaria* and regarded as cryptopine has been further identified as such by comparison with an authentic specimen from opium which was kindly supplied by Dr. R. D. Haworth of Armstrong College, Newcastle-upon-Tyne.

The sixth alkaloid was obtained in such minute amounts that there was insufficient even for a microanalysis. It melts sharply at 201° C. and has the appearance of homogeneity. It is proposed to refer to this substance as alkaloid γ until further characterization is possible.

Experimental

The procedure outlined in some detail (4) in a communication dealing with *Adlumia fungosa* has been strictly followed. There was available a total of 3850 gm. of dried plant material of which the woody tap roots constituted 325 gm. The quantity of total alkaloid from the latter was sufficient only for the isolation and purification of protopine and bicuculline, and the mother liquors from these were combined with the corresponding fractions from the aerial portion. In the following record the designations of the various fractions are the same as those previously given (4).

Isolation of Fumaric Acid

This acid was isolated in precisely the same manner as from *A. fungosa*. Comparison with an authentic specimen as well as with the acid from the above source proved its identity. After appropriate purification it melted and sublimed at 295° C. About 4 gm. was obtained from the roots and an equal amount from the stems and leaves.

Isolation of Capnoidine, $C_{19}H_{17}O_6N$(?)

The chloroform extract (C) was freed of solvent as far as possible on a steam bath and then evaporated several successive times with methanol. Crystallization of a sparingly soluble hydrochloride was complete after several days. The substance was filtered off, washed with cold methanol and recrystallized by the addition of methanol to a concentrated aqueous solution. After filtering and washing, the hydrochloride (m.p., 244° C.) as thus obtained was treated with excess potassium hydroxide in aqueous solution. The precipitate which rapidly crystallized was filtered off and thoroughly washed with water. It then melted at 233° C. Recrystallization was readily effected by adding hot methanol to a concentrated chloroform

solution of the base, and yielded brilliant stout prisms of *capnoidine* which melted sharply at 235° C. in spite of the development of a slight brown color at a temperature a few degrees lower. Calcd. for $C_{19}H_{15}O_6N$; C, 64.59; H, 4.26; N, 3.97%. Mol. wt., 353. Calcd. for $C_{19}H_{17}O_6N$; C, 64.23; H, 4.79; N, 3.94%. Found: C, 64.94, 65.14; H, 4.70, 4.69; N, 4.07, 3.95%. Mol. wt., 337, 338 (Rast). Methoxyl, negative.

An intimate mixture of capnoidine and adlumidine began to sinter at 206° and was completely liquid at 212° C.

Isolation of Bicuculline and Bicucine

The combined mother liquors from the capnoidine were freed of methanol, the residue treated with hot dilute hydrochloric acid, filtered from some resin and thoroughly exhausted with ether. The aqueous solution (ASR) on appropriate treatment yielded a small amount of a base (BC) which proved on recrystallization to consist largely of the sparingly soluble capnoidine together with a small amount of bicuculline which was isolated from the mother liquors.

The ether extract (EC) yielded a few crystals of protopine (melting point and mixed melting point, 211° C.). It may be pointed out that protopine like allocryptopine is not completely precipitated by means of fixed alkali and it has repeatedly been obtained in appreciable amounts by extracting the aqueous filtrate with ether.

The precipitate (BCE) obtained by carbonating the alkaline filtrate (CES) was dried in a desiccator and extracted with chloroform. The filtrate was evaporated to a small volume and treated with a little methanol. In the course of several days about 0.5 gm. of almost colorless stout prisms separated, which after one recrystallization from chloroform-methanol melted sharply at 177° C. It was observed for the first time in this case that the resolidified melt on reheating melted at 195° C. Admixture with a specimen of bicuculline melting at 177° C. from *D. cucullaria* caused no depression in melting point, and the hydrochlorides from both sources exhibited identical properties.

The fraction of (BCE) which remained insoluble in chloroform was suspended in concentrated ammonia solution and a stream of gaseous ammonia passed in until only an insignificant amount of slimy residue remained. The latter was removed with the aid of charcoal and the filtrate placed in an evacuated desiccator over concentrated sulphuric acid. This is the only procedure which in the writer's experience will yield well-defined crystals of bicucine. Crystallization was slow but complete and the stout brilliant prisms thus obtained melt sharply at 217° C. When admixed with a specimen of alkaloid (β) (2) melting at 215° C. obtained from *D. cucullaria* by carbon dioxide precipitation from an alkaline solution, the mixture melted at 215° C. The yield was 0.7 gm.

Since previous analyses had not yielded figures which admitted of an unequivocal interpretation, this specimen was analysed after drying in a high vacuum over phosphorus pentoxide for 48 hr. during which time it lost about 1% of its weight. The combustion figures on the dried sample are in fair

agreement with the monohydrate of $C_{20}H_{19}O_7N$. This representation is preferred to the empirical formula $C_{20}H_{21}O_8N$. Calcd. for $C_{20}H_{21}O_8N$; C, 59.55; H, 5.21; N, 3.47; NMe, 3.72%. Found: C, 60.29; H, 5.22; N, 3.58; NMe, 3.86%. (Means of duplicates). (Herzig-Meyer). Methoxyl, absent.

Isolation of Protopine

Almost the entire quantity of alkaloids in the chloroform extract (AC) was obtained in the non-phenolic fraction (BS). The other fractions consisted for the greater part of insignificant residues largely contaminated with non-basic resins. Much larger quantities of plant material are necessary to permit of the isolation of appreciable amounts of pure products from these fractions.

The dried mixture of bases (BS) was dissolved in chloroform and a turbidity removed with charcoal. The filtrate was evaporated somewhat, treated with an equal volume of methanol and the solution again boiled with charcoal. This twofold treatment with charcoal is one that has repeatedly demonstrated its merits. Solutions of alkaloids obtained from precipitates or from aqueous solutions by means of immiscible solvents invariably contain small amounts of inorganic matter which is effectively removed by filtering a chloroform solution with the aid of charcoal. This treatment does not eliminate colored impurities appreciably. It has been found however that the second treatment with charcoal in the presence of as much methanol as feasible is very effective in removing color.

The filtrate from the second charcoal treatment was slowly evaporated on a steam bath to a thin syrup, which rapidly deposited large almost colorless stout prisms of protopine when it was seeded. After one recrystallization from chloroform-methanol the protopine was obtained in brilliant colorless crystals which alone or admixed with specimens of pure protopine from a number of other fumaraceous plants melted at 211° C. The mother liquors were again treated with charcoal and yielded a further amount, the total quantity obtained being 2.8 gm.

Isolation of Cryptopine and Alkaloid (γ)

The mother liquors from which no more protopine could be crystallized were all combined, evaporated to a small volume and acidified with a slight excess of concentrated hydrobromic acid in methanol. A small amount of protopine hydrobromide crystallized out in the course of several days. The filtrate from this was freed of methanol, the bases regenerated, and extracted with ether. The solvent was removed from the extract and the residue dissolved in an excess of aqueous oxalic acid. In the course of several days a crop of sparingly soluble crystals separated. This product was filtered off, washed with a little water and the base liberated by means of an excess of potassium hydroxide. It was extracted with a large volume of ether and the residue from the extract recrystallized from hot methanol. Except for an insignificant amount in the mother liquor the entire product was obtained in fine colorless prisms melting sharply at 221° C. Admixture with a specimen of cryptopine from opium or with one from *D. cucullaria* did not depress this

melting point. Color reactions of the bases from the three sources carried out side by side failed to show any differences. The total yield of cryptopine was 0.05 gm.

The mother liquor from the crystallization of the cryptopine oxalate was basified with excess potassium hydroxide and the liberated base removed with ether. The residue from the ether extract crystallized completely in contact with a little methanol. The crystals consisted of short stout prisms and appeared to be homogeneous; m.p., 201° C. The yield was somewhat less than 0.01 gm. Until this base is available in sufficient quantity for adequate characterization it will be referred to as alkaloid (γ).

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REVIEWS AND NOTES

A METHOD FOR FINDING SMALL LEAKS IN A HIGH-VACUUM APPARATUS[1]

By F. R. Terroux[2] and W. H. Watson[3]

It is well known that the usual methods for finding a leak in a high vacuum system are of little use when the rate of leak is of the order of 10^{-4} mm. per hour or less, and arises from invisible defects in a metal-to-glass seal. Anyone who has experienced such leaks will admire the restraint with which Dunoyer (2, p. 154) describes the situation; "La recherche..... constitue un problème toujours fastidieux, occasion de grandes pertes de temps et parfois même insoluble". Obviously it may be possible to test each seal separately in a system of small capacity before assembly, but in spite of this, leaks of this magnitude may develop after the seals have been joined to the complete vacuum system.

The method here described makes use of the principle that the rate of leak depends on the difference of pressure prevailing across the fault. It provides a very simple technique for reducing the pressure on the outside of a seal, such as a pinch, without damaging the lead-in wires. When the pressure on the outside of a faulty seal is reduced in this way, the rate of leak of the evacuated apparatus will be markedly diminished, and if this is the only defective seal the rate of leak will become negligible. Clearly this technique can be applied to each seal in turn until the defective one is identified, and measures can be taken to repair or replace it.

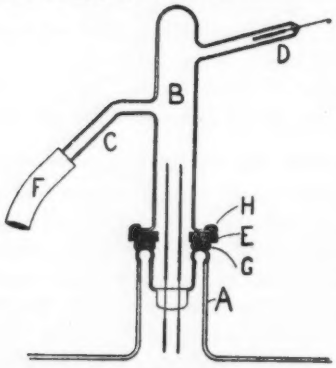

Fig. 1. *Application of the method to a typical seal.*

Reference to Fig. 1 will make clear the method actually used. The seal to be tested is shown at *A*. *B* is a tube of glass, or Pyrex, about 15 cm. long and 2 cm. in diameter with a flange about 1 cm. wide at the bottom. The side tube *C* serves to connect *B* to some form of vacuum pump. An electrode is sealed into the side tube *D* and the pressure in *B* can be estimated from the nature of the discharge when the electrode is connected to a small induction coil. The joint between the flanged end of *B* and the rim of seal *A* is made by using a rubber washer *E*, about 6 mm. thick, pressed firmly against a layer of plasticene* *G* which has been worked smoothly

[1] *Manuscript received February 24, 1933.*
Contribution from the Department of Physics, McGill University, Montreal, Canada.
[2] *Senior Demonstrator, Department of Physics, McGill University.*
[3] *Assistant Professor, Department of Physics, McGill University.*

* *Plasticene was used with great success by Cockcroft and Walton (1) in the construction of high voltage positive ray tubes.*

into an annular shape before assembly. The joint is completed by pressing, with the fingers, another ring of plasticene *H* around the *outside* of the flange at the junction of *B* and *E*.

In practice, if the whole system is gently pressed together by hand and the pump started, then, provided the rubber connecting tube *F* is properly supported, the pressure in the space enclosed between the seal *A* and the tube *B* will rapidly be reduced to a few hundredths of a millimetre. This pressure is maintained by keeping the pump in operation. All that remains is to measure the rate of leak in the main vacuum system over an interval of a few hours. If the rate of leak is less than it was when the outside of *A* was open to the atmosphere, then seal *A* is the seat of the trouble. While this technique may be adapted to almost any conditions, the use of re-entrant seals in vacuum systems makes this method of detecting leaks very easy to apply.

In conclusion, the success of the above method suggests the idea that when a vacuum system is to be sealed off at very low pressure and is to retain its vacuum over a very long period, the use of double seals with the space between highly evacuated would give far greater protection at all metal-to-glass seals. Thus any ordinary seal which shows no appreciable leak in 24 hr. when exposed to atmospheric pressure, would, when backed by an evacuated space (and excluding accidents) show no appreciable leak over a period of many months.

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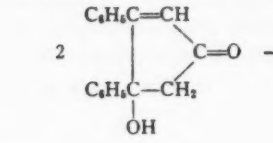
THE STRUCTURE OF THE DIKETONE OBTAINED FROM ANHYDRACETONEBENZIL[1]

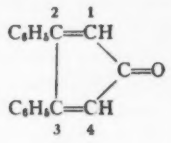
By C. F. H. Allen[2] and E. W. Spanagel[3]

In a recent paper the writers (1) described the formation of a bimolecular product, $C_{34}H_{24}O_2$, from anhydracetonebenzil in various reactions. This substance had been made previously by Japp* who found that on being heated it lost carbon monoxide, with consequent formation of a new compound, $C_{33}H_{24}O$, that formed a phenylhydrazone. Japp did not suggest a structure for either of these compounds.

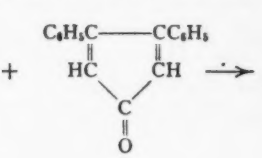
In the writers' previous paper (1, p. 4340) the assumption was made that the dehydration of anhydracetonebenzil (I) gave a cyclopentadienone (II), two molecules of which then reacted to produce the bimolecular product. Bearing in mind the addition reactions of Diels and Alder, it was assumed that to form the bimolecular product one molecule of the cyclopentadienone added to the ends of the conjugated system (1 : 4) in the second, yielding a tricyclic system containing a carbon monoxide bridge (III). On being heated

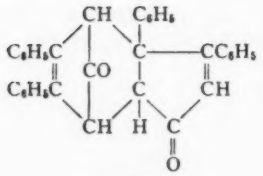


(I)

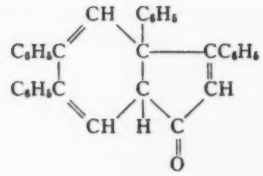


(II)

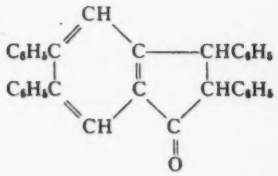




(III)



(IV)



(V)

the carbon monoxide bridge could be split out, leaving a ketone (IV). Two isomeric ketones have been found, one of which gave a phenylhydrazone agreeing with Japp's; the second was formed by heating the first longer or to a higher temperature, and is a hydrindone (V).

The structure of the latter was carefully determined by a long series of degradation reactions that terminated in *o*-diphenylbenzene, showing that substance (III) must contain this skeleton.

The writers have also found that tetraphenylcyclopentadienone adds maleic anhydride to form a bicyclic system having a carbon monoxide bridge.

Reference

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[1] *Manuscript received March 21, 1933.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Demonstrator in Chemistry, McGill University.*
* *References given in paper cited above.*